

THE ALARM – DEFENCE SYSTEM IN FORMICINE ANTS

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The thesis is a summary of the following papers:

- I. Chemical congruence of the complex odoriferous secretions from Dufour's gland in three species of ants of the genus Formica. Bergström G. and Löfqvist J. 1973. - J. Insect Physiol. 19: 877-907.
- II. Apparatus for automatic indicating of the activity of organisms, especially animals. Stigmark K., Jonsson I., Löfqvist J., and Stenram H. 1972. - United Kingdom Patent No. 1265614. The Patent Office, London.
- III. Formic acid and saturated hydrocarbons as alarm pheromones for the ant Formica rufa L. Löfqvist J. 1976. - J. Insect Physiol. in press.
- IV. Toxic properties of the chemical defence system in Formica rufa L. and Formica sanguinea Latr., two competitive species. Löfqvist J. 1976. -

Background

JEAN HENRI FABRE, the famous French naturalist, wrote in his *Souvenirs entomologiques* (1879 - 1907) "que la science, instruite par la bête, nous dote un jour du radiographe des odeurs, et ce nez artificiel nous ouvrira tout un monde de merveilles."

FABRE realized the importance of odours for communication in many insect species. It was, however, first in the late 1950's that more substantial progress was made. This necessitated a new term for communication substances. KARLSON and LUSCHER (1959) introducing the term "pheromone" wrote "Pheromones are defined as substances which are secreted to the outside by an individual and received by a second individual of the same species, in which they release a specific reaction, for example, a definite behaviour or a developmental process." In the same year the first insect pheromone was chemically identified, viz. a hexadeca-dien-(10, 12,)-ol(1) (BUTENANDT et al. 1959). The new field of research has since then grown rapidly.

There are two reasons for the development of pheromone research. Firstly, the introduction of chemical analytical methods for analysis of minute amounts (10^{-6} - 10^{-9} g) of operating pheromones. The advance of gas chromatography during the middle and late 1950's and the combination of gas chromatographic and mass spectrometric techniques in late 1950's were of crucial importance.

Secondly, ethology developed as an independent branch of science. Odours as communication signals, especially in insects, became an important area of research from a general biological point of view as well as in applied entomology as a possible method for biological control of insect pests. The advantages of such control methods are evident. They are based on communication systems which are species or species-group specific.

Present knowledge of insect pheromones has been summarized in handbooks e.g. BIRCH 1974, JACOBSON 1972, SONDHEIMER and SIMEONE 1970 and in reviews, e.g. BLUM 1969, KAISSLING 1971, and PRIESNER 1973.

Communication in ant species

Social insects for their life in nests inhabited by hundreds or thousands of

individuals need sophisticated communication systems. In the excellent books "The insect societies" (1971) and "Sociobiology: The new synthesis" (1975) E.O. WILSON demonstrated that ants need a communication system mainly in four situations:

1. Alarm-defence. Attacked individuals communicate to other individuals that an enemy is threatening the nest, the superorganism. A massive mobilization - for defence or flight - can in this way be rapidly effected.
2. Recognition. Individuals in a society recognize each other as well as ants from hostile nests.
3. Orientation. Information on food sources far away from the nest is communicated to other nest individuals by odour trails.
4. Sex attraction and recognition.

Various steps in these four behaviour patterns are communicated by odours. Among ants the chemical sense is unusually highly developed.

This thesis deals with the alarm-defence system in some formicine ants, e.g. stingless ants.

Odour glands in formicine ants

In formicine ants the poison gland consists of two coiled filaments lying as a disc on the top of the large poison vesicle. The gland opens at the tip of the gaster. It produces only formic acid in a concentration of about 60 % (cf. references in I and IV).

A small gland, Dufour's gland, opens in the duct of the poison vesicle. Its contents are probably always emptied together with formic acid from the poison vesicle (cf. references in I). That Dufour's gland is a perfume factory in Hymenoptera was first shown by CAVILL and WILLIAMS (1967) for an ant species, Myrmecia gulosa (F.), and for two formicine species, Formica sanguinea Latr. by BERGSTRÖM and LÖFQVIST (1968) and Acanthomyops claviger (Roger) by REGNIER and WILSON (1968). In formicine worker ants the gland was found to produce most of the odour substances present in the ants. It was later shown

that the gland is a rich source for odour substances also in other hymenopter species such as bumble bees and bees (cf. KULLENBERG and BERGSTRÖM 1975 and references therein).

In some formicine ants odour trails are laid with substances from the hind gut. This has been investigated most thoroughly in Lasius fuliginosus Latr. in which the trail pheromones have been identified as a series of fatty acids (HUWLYER et al. 1975).

Four glands open in the mouth region of formicine ants: the pharyngeal glands, the labial glands, the maxillar glands, and the mandibular glands. The pharyngeal glands and labial glands possibly have digestive functions. Their role and the chemistry of their secretions are, however, unknown which also applies to the maxillar glands. The mandibular glands in worker ants are in some species e.g. Acanthomyops claviger (CHADHA et al. 1962) and Lasius umbratus (Nyl.) (BLUM et al. 1968) known to produce odour substances, mainly isoprenoids which function as alarm-defence substances. BERGSTRÖM and LÖFQVIST (1970) identified some substances from the heads of four Lasius species.

The mandibular glands of males of some formicine ants contain a wide spectrum of volatile substances (BERGSTRÖM and LÖFQVIST unpubl.), as in male bumble-bees and bees. The chemistry of the gland secretion from the latter has been thoroughly investigated (BERGSTRÖM 1973).

The metapleural glands in formicine ants open on metathorax. Their secretion is antiseptic and probably protects the ants and their nests from microorganisms (MASCHWITZ et al. 1970, MASCHWITZ 1974).

The number of glands and compounds illustrate the dependence of formicine ants on chemical communication.

Methods for chemical analysis of the gland secretions

Three modifications of the same method have been used for isolating and concentrating the odour substances: 1. Vacuum distillation of hundreds of whole worker ants. 2. Vacuum distillation of a few ant gasters. 3. Distillation of excised Dufour glands.

In the first two cases the substances were collected in a cool trap and washed with an organic solvent. In the third case they were transferred directly to the gas chromatographic column (cf. STÄLLBERG-STENHAGEN 1972, BERGSTRÖM 1973).

Separation and chemical identification of the substances have been performed by gas chromatography with packed columns as well as with glass capillary columns in connection with a flame ionization detector. The main method for identification was mass spectrometry. A combined gas chromatograph-mass spectrometer has been used. In a few cases high-resolution mass spectrometry has also been utilized.

Chemically investigated material

Odour substances have been chemically identified by BERGSTRÖM and LÖFQVIST (1968, 1970, 1972 a, 1972 b, 1973) from worker ants of the following species: Camponotus herculeanus (L.), C. ligniperda (Latr.), Lasius niger (L.), L. alienus (Foerst), L. flavus (F.), L. carnolicus Mayr., Formica sanguinea Latr., F. rufa L., F. polycтена Foerst., F. nigricans Em., F. rufibarbis Fabr., F. fusca L., and Polyergus rufescens (Latr.).

The odorous substances dealt with in the present thesis emanate from Dufour's gland in worker ants of F. nigricans, F. rufa, and F. polycтена.

Chemical composition of the volatile secretion from Dufour's gland in formicine ants

The chemical composition of the volatile secretion from Dufour's gland has been studied in a few species (BROPHY et al. 1973, REGNIER and WILSON 1968, 1969) besides those analysed by BERGSTRÖM and LÖFQVIST (op. cit.). The results from all investigated species can be summarized as follows :

1. The gland secretion usually consists of more than ten substances.
2. The substances cover a wide range of volatility from high volatile hydrocarbons with nine or ten carbon atoms to low volatile substances with more than 20 carbon atoms.

3. Characteristic for most species is the presence of a homologues series of aliphatic, saturated hydrocarbons. Undecane is the predominating substance often making up about half of the secretion. There is also in many species one or more homologous series of mono-unsaturated, doubly unsaturated, or methyl branched hydrocarbons, usually present in trace amounts only.
4. The secretion contains usually one or several homologous series of aliphatic, saturated or mono-unsaturated acetates, ketones or alcohols. The substances of one of these series are usually present in considerable amounts giving the secretion a characteristic composition especially in the median and low volatile part.
5. In many species there are also one or a few isoprenoids. These substances are always present in small or trace amounts only.
6. The total amount of the secretion from Dufour's gland is about 0.05 mg in worker ants of the Formica species investigated.

Quantitative methods for studies of the alarm behaviour in ants

By measuring the strength of the stimulus and the intensity of the behavioural response the alarm behaviour can be evaluated quantitatively.

The strength of the stimulus has in this thesis been estimated in molecules saturated gas of the test substance according to KAFKA (1970).

Many "releaser pheromones" in insects cause a strong excitement in other individuals which start running around quickly. The intensity of this reaction has been studied by measuring the locomotor activity. The method has been developed by Research Engineer INGVAR JONSSON and Professor LENNART STIGMARK at Lund Institute of Technology in co-operation with myself and Mr. HÅKAN STENRAM. It is described in II.

The method utilizes the capacity variations of a condenser influenced by moving insects. The variations are related to the locomotor activity in such a way that there is a direct linearity. The instrument has been designed for measurements of walking animals under laboratory conditions, but it can also be used in other situations.

The measuring method has the following advantages:

1. The locomotor activity is registered automatically without disturbing the animals. The weak electrical field does not, as far as is known, influence the insects,
2. If required, small movements (cleaning, etc.) which are not locomotory can be excluded.
3. The instrument has a very high electrical stability. Continuous registrations can be made for days and weeks making determination of the diurnal rhythm of the animals possible.
4. Environmental conditions (temperature, humidity, light, wind velocity, etc.) can be changed without influencing the registering system.
5. Small (about 1 mm) as well as large terrestrial animals can be studied.
6. The locomotor activity of many individuals can be examined at the same time. This is important for studies of social and semisocial animals.

Biological action of alarm pheromones in *Formica rufa*

The alarm-defence weapon in *F. rufa* consists of formic acid from the poison vesicle mixed with the secretion from Dufour's gland. The gland was found to contain 41 substances, most of which were hydrocarbons. A homologous series of aliphatic, saturated hydrocarbons made up about 85 % of the secretion with undecane alone forming about 50 % (I).

The hydrocarbons from octane and up to tridecane were investigated as releasers for the alarm behaviour in *F. rufa* as pure substances as well as in combination with formic acid (III). Tridecane and dodecane were found to be weak alarm pheromones, while the other hydrocarbons released an intense alarm behaviour. The behavioural threshold for decane was lower than $5 \cdot 10^{13}$ molecules cm^{-3} air. Pure formic acid is a strong alarm pheromone for *F. rufa*. Its threshold value was estimated to $7 \cdot 10^{15}$ molecules cm^{-3} air.

Electrophysiological studies (KAFKA and NEUWIRTH 1975 and references therein) have shown that odour substances can be grouped in "reaction groups". The components of such a group are suggested to affect a specific kind of acceptor inside the receptor. The hydrocarbons tested seem to belong to the

same reaction group, while formic acid was shown to belong to another reaction group.

The total intensity and the duration of the alarm behaviour released by formic acid and one hydrocarbon are additive if the two substances are combined in one stimulus. A combination of two hydrocarbons and formic acid releases a stronger reaction than one hydrocarbon plus formic acid even if the two stimuli contain the same number of molecules. The hydrocarbons have a combined effect and their relative concentrations determine the intensity and duration of the alarm behaviour. This regulating ability of the hydrocarbons is a result of their volatility. The role of the broad range of volatility may explain the presence of series of substances in pheromone secretions.

Toxic properties of the defence system in *Formica rufa* and *F. sanguinea*

F. sanguinea is a slave-keeping ant. It raids nests mainly of *F. fusca* and *F. rufibarbis* for cocoons, which are taken to its own nest where, after emergence, the alien ants serve as slaves and carry out much of the work.

F. rufa and *F. sanguinea* are nest competitors. *F. sanguinea* seems to be the superior and sometimes takes over vigorous *F. rufa* nests. The defence system of the two consists of different substances. The toxic properties of the defence systems have been studied, including the apparent competitive advantage of *F. sanguinea* over *F. rufa* (IV).

Formic acid and substances from Dufour's gland constitute the chemical defence system in *F. rufa* and *F. sanguinea*. The secretion from Dufour's gland consists in *F. rufa* mainly of hendecane and tridecane and in *F. sanguinea* mainly of hendecane, decyl acetate, and dodecyl acetate. At least the hydrocarbons have been shown to be toxic for the ants also in small amounts. The most important role of the hydrocarbons and the acetates is, however, as wetting agents for formic acid, which by itself is fairly harmless. There are only slight differences between the hydrocarbons and the acetates as wetting agents for formic acid. The substances kill the ants by penetrating their tracheal system, thus easily reaching the vital organs.

The hydrocarbons and formic acid of *F. rufa* are more toxic for both spe-

cies than the substances of F. sanguinea. F. sanguinea also seems to be more sensitive to the defence substances from both the species than F. rufa. The reason may be its smaller size.

Differences in the composition of the secretion of Dufour's gland in the two species do not explain the superiority of F. sanguinea in the nest competition with F. rufa observed in the field. It may be explained by higher aggressiveness in F. sanguinea than in F. rufa.

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CHEMICAL CONGRUENCE OF THE COMPLEX ODORIFEROUS SECRETIONS FROM DUFOUR'S GLAND IN THREE SPECIES OF ANTS OF THE GENUS *FORMICA*

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Abstract—Forty-six volatile compounds were identified in the secretions from Dufour's gland of worker ants of the species *Formica nigricans*, *F. rufa*, and *F. polyctena* using combined gas chromatography-mass spectrometry and capillary gas chromatography. In both methods the natural volatile material has been driven off excised glands using a precolumn technique.

The composition of the secretions from the three species are very similar but there are also some distinct differences, especially between *F. nigricans* on the one hand and *F. rufa* and *F. polyctena* on the other. Straight-chain, saturated hydrocarbons are the quantitatively dominating compounds, with undecane as the largest individual component, followed by tridecane, pentadecane, and heptadecane. Straight-chain, monounsaturated hydrocarbons and methyl-branched, saturated hydrocarbons are also present and a few straight-chain, doubly unsaturated hydrocarbons are present in trace amounts.

In the region of lower volatility two isoprenoids identified as all-*trans*-geranylgeraniol and the corresponding acetate all-*trans*-geranylgeranyl acetate, have been found together with octadecyl acetate. The relative amounts between these compounds are the main difference between the secretions from the three species. Octadecyl acetate is thus present only in trace amounts in the secretion of *F. nigricans*.

In addition to the compounds mentioned a few aliphatic acetates and alcohols present in small amounts have been identified.

The secretions are thought to have many functions which are reflected in their complex composition. This is discussed in comparison with results from other formicine ants. The species specificity in the composition of the secretion from Dufour's gland and the taxonomic value of this specificity are also discussed.

INTRODUCTION

WHEN attacked most worker ants of the genus *Formica* respond by spraying their enemy. The spray is a mixture of formic acid from the poison vesicle and the content of Dufour's gland.

Dufour's gland in *Formica* is a small gland located in the tip of the gaster. This gland was first described by DUFOUR (1841) from bees, bumblebees, wasps, etc. It is now known to be present in all females and workers of Hymenoptera.

In a recent paper ROBERTSON (1968) summarized the literature and also investigated the gland in the major groups of the Hymenoptera. HERMANN and BLUM (1968) have investigated the whole poison apparatus in *Camponotus pennsylvanicus*.

As shown in an excellent work by FOREL (1878), Dufour's gland opens in the short duct of the poison vesicle. FOREL investigated several formicine ants especially *F. rufibarbis*. The contents of Dufour's gland are discharged together with formic acid from the poison vesicle. It is still an open question if formicine ants really can emit substances from Dufour's gland separately.

It is obvious that formic acid is a defence substance for formicine ants. GHENT (1961) was the first to point out that the penetration of formic acid was in a high degree facilitated by lipoid substances which lowered the surface tension of the acid. Such substances from Dufour's gland are very important for spreading the acid over the cuticle of an enemy.

That the substances from Dufour's gland in formicine ants also are important as alarm pheromones was shown by MASCHWITZ (1964), in a thorough investigation. Recent reviews of alarm pheromones in ants are those by BLUM (1969) and WILSON (1971). In this fourth paper we have analysed the contents of Dufour's gland in worker ants of *F. nigricans*, *F. rufa*, and *F. polyctena*.

Already a general gas chromatographic analysis of the content of Dufour's gland from many ants shows that the secretion is made up of more than 30 different chemical substances. It is true that many of these are only present in trace amounts but this does not absolutely mean that they do not have any biological importance.

Taxonomy

The systematics within the European *F. rufa* group in its widest sense is generally considered as confused. The reason for this situation is that within the group there are many pairs of closely related forms which are regarded as species (YARROW, 1955; LANGE, 1958; BETREM, 1960). It is therefore necessary to explain which forms of the ants we have been working with.

F. nigricans Em., one of the species now analysed, is part of such a species pair within which the species names have varied a great deal during the last years. We use here the name proposed by European myrmecologists when they got together in Würzburg in 1963 (ANONYMOUS, 1965).

Both BETREM (1960) and KUTTER (1965) have pointed out that *F. nigricans* Em. can be separated from the closely related *F. pratensis* Retz. only on the pilosity of the queens. We have studied the pilosity of 57 newly emerged queens taken from the same nest as all the analysed worker ants. The results strongly indicate that the form we have analysed is *F. nigricans* Em.

The two other species now analysed, *F. rufa* L. and *F. polyctena* Förster, form another species pair. They are separated mainly on the workers which in *F. rufa* are hairy, especially on the thorax, but in *F. polyctena* they are almost naked (BETREM, 1960). The variation in this and other characteristics of both species is so wide that they largely overlap each other. For the analyses we have, however, chosen nests with ants of a very typical appearance.

MATERIALS AND METHODS

Collection of ants

Ants were always collected from only one nest for each species (except for two analyses of minor importance in *F. polycтена*, cf. Table 4). The reason for this is the poor taxonomy of the ants. When we began we therefore chose one nest for each species with ants of a morphology very typical for the species.

The three ant nests were all situated in the vicinity of the Ecological Station in Öland, an island in the southern Baltic. The ants were always carefully picked up with a stick one by one and then chilled to 4°C and stored at this temperature until they were used. The dates for the different collections are given in Tables 1, 3, and 4.

Analysed material and isolation of volatile components

For *F. rufa* and *F. polycтена* the investigation was initiated with a sample of 300 whole ants (cf. Tables 3 and 4) which were vacuum degassed. The reason for this was to obtain a picture of the odour spectrum from the whole ant. For all the following analyses of the two species as well as for all the analyses of *F. nigricans* we have only used excised Dufour glands. We have only used whole and filled glands. The number of glands used in each analysis is shown in Tables 1, 3, and 4.

Because there are very few drawings of Dufour's gland in formicine ants we show in Fig. 1 the morphology, size, and position of the gland in the gaster of a worker ant of *F. rufa*. The morphology and relative size of the gland of *F. nigricans* and *F. polycтена* is quite similar.

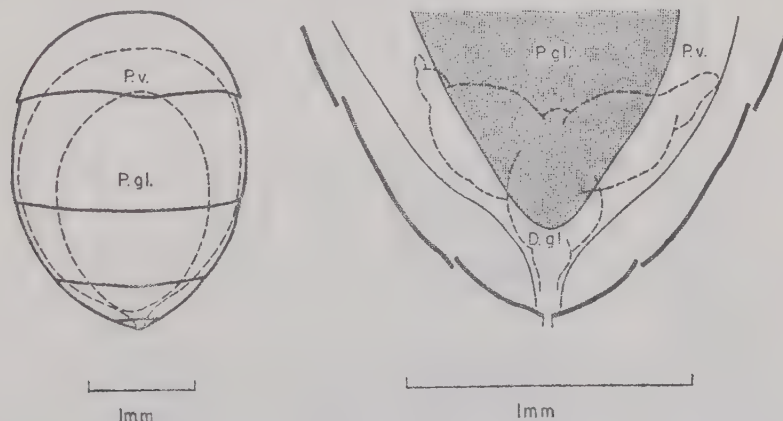


FIG. 1. Schematic drawings of the gaster from the dorsal side in worker ants of *F. rufa* showing to the left the size and position of the poison vesicle (P.v.) with the poison gland (P.gl.) and to the right Dufour's gland (D.gl.). This gland is situated below the poison vesicle in the duct of which it opens. The spheric size of the poison vesicle is as the size of the poison gland, but *in situ* it flows out more or less as a disk over the internal organs.

TABLE 1—ANALYSED MATERIAL OF *F. nigricans*, WORKERS

Analysis No.	Date of collection	Locality	Part of the body	No. of ants	Isolation method	Analytical method	Date of analysis
1	9 Sept. 1969	Öl. Skogsby	Dufour's gland	62	Ether extract	GC/MS	9 Sept. 1969
2	9 Sept. 1969	Öl. Skogsby	Dufour's gland	62	Ether extract	GC	10 Sept. 1970
3	16 Sept. 1970	Öl. Skogsby	Dufour's gland	30	Precolumn	GC/MS	16 Sept. 1970
4	18 Aug. 1971	Öl. Skogsby	Dufour's gland	20	Ether extract	CGC	19 Aug. 1971
5	18 Aug. 1971	Öl. Skogsby	Dufour's gland	3	Precolumn	CGC with references	24 Aug. 1971
6	18 Aug. 1971	Öl. Skogsby	Dufour's gland	5	Precolumn	CGC	27 Aug. 1971
7	15 Oct. 1971	Öl. Skogsby	Dufour's gland	1	Precolumn	CGC with references	19 Nov. 1971
8	15 Oct. 1971	Öl. Skogsby	Dufour's gland	12	Precolumn	GC/MS	19 Nov. 1971
9	15 Oct. 1971	Öl. Skogsby	Dufour's gland	1	Precolumn	CGC	2 Dec. 1971
10	15 Oct. 1971	Öl. Skogsby	Dufour's gland	1	Precolumn	CGC with references	6 Dec. 1971
11	15 Oct. 1971	Öl. Skogsby	Dufour's gland	1/2	Precolumn	CGC with geranyl-geraniol	6 Dec. 1971
12	7 May 1972	Öl. Skogsby	Dufour's gland	20	Hexane extract	GC/MS, ozonolysis	16 June 1972

GC, Gas chromatography with packed glass columns; CGC, capillary gas chromatography; GC/MS, combined gas chromatography/mass spectrometry.

TABLE 3—ANALYSED MATERIAL OF *Formica rufa*, WORKERS

Analysis No.	Date of collection	Locality	Part of the body	No. of ants	Isolation method	Analytical method	Date of analysis
1	28 Aug. 1967	Öl. Skogsby	Whole ants	300	Vacuum degassing	GC/MS	10 Oct. 1967
2	6 Sept. 1969	Öl. Skogsby	Dufour's gland	20	Ether extract	GC/MS	7 Sept. 1969
3	6 Sept. 1969	Öl. Skogsby	Dufour's gland	1	Ether extract	GC	7 Sept. 1969
4	6 Sept. 1969	Öl. Skogsby	Dufour's gland	1	Ether extract	GC	7 Sept. 1969
5	6 Sept. 1969	Öl. Skogsby	Dufour's gland	1	Ether extract	GC	7 Sept. 1969
6	6 Sept. 1969	Öl. Skogsby	Dufour's gland	1	Ether extract	GC	7 Sept. 1969
7	6 Sept. 1969	Öl. Skogsby	Dufour's gland	1	Ether extract	GC	7 Sept. 1969
8	10 Sept. 1969	Öl. Skogsby	Dufour's gland	53	Ether extract	GC/MS	11 Sept. 1969
9	10 Sept. 1969	Öl. Skogsby	Dufour's gland	53	Ether extract	GC	11 Sept. 1969
10	16 Sept. 1969	Öl. Skogsby	Dufour's gland	1	Ether extract	GC	17 Sept. 1969
11	16 Sept. 1969	Öl. Skogsby	Dufour's gland	1	Ether extract	GC	17 Sept. 1969
12	16 Sept. 1969	Öl. Skogsby	Dufour's gland	1	Ether extract	GC	17 Sept. 1969
13	5 Nov. 1969	Öl. Skogsby	Dufour's gland	1	Precolumn	GC	5 Nov. 1969
14	2 Sept. 1970	Öl. Skogsby	Dufour's gland	3	Precolumn	GC	2 Sept. 1970
15	14 Sept. 1970	Öl. Skogsby	Dufour's gland	40	Precolumn	GC/MS	14 Sept. 1970
16	17 Aug. 1971	Öl. Skogsby	Dufour's gland	1	Precolumn	CGC	24 Aug. 1971
17	17 Aug. 1971	Öl. Skogsby	Dufour's gland	3	Precolumn	CGC with references	24 Aug. 1971
18	21 Sept. 1971	Öl. Skogsby	Dufour's gland	5	Precolumn	CGC	21 Sept. 1971
19	11 Oct. 1971	Öl. Skogsby	Dufour's gland	4	Precolumn	CGC	11 Oct. 1971

GC, Gas chromatography with packed glass columns; CGC, capillary gas chromatography; GC/MS, combined gas chromatography/mass spectrometry.

Some samples were extracted in diethyl ether and injected on the gas chromatographic columns. The most superior method was, however, to use a precolumn from which the volatile substances were transferred directly on to a gas chromatographic column. With this method it is possible to work with very small amounts of biological material. The precolumn technique has been described for packed columns by BERGSTRÖM (1973) and for capillary columns by STÄLLBERG-STENHAGEN (1972). The number of analyses and data for each of them are given in Tables 1, 3, and 4.

The precolumn used in combination with the capillary gas chromatography column functions in fact as a splitter-free intake system. Therefore the sensitivity of the gas chromatograph is retained. In this way volatile compounds from glands have been driven into the separation column. When reference material has been added, the solvent used (hexane) has first been allowed to evaporate in the open air.

Gas chromatography and mass spectrometry

Two gas chromatograph-mass spectrometer combination instruments, one LKB-9000 available at the Ecological Station, Ölands Skogsby and one built at the Institute of Medical Biochemistry, Göteborg, were used in this work. The gas chromatographic columns used in the combination instruments utilized Silicone SE-30 'Ultraphase', 2% on Chromosorb G 80-100 mesh, or Silicone SF 96, 4% on Anacrome 70-80 mesh as a stationary phase. The electron energy was kept at 70 eV in all analyses.

One Perkin-Elmer 900 and one Perkin-Elmer 990 gas chromatograph equipped with precolumns (*cf.* STÄLLBERG-STENHAGEN, 1972) were used together with capillary columns. A Silicone OV-101 stationary phase was used for a 23 m long glass capillary column (our No. 66) together with the first mentioned instrument. This column had an efficiency of 88 000 theoretical plates, measured for methyl pentadecanoate. The other instrument was used with another glass capillary column (our No. 55) coated with OV-1 Silicone as the stationary phase. This 28 m long column gave 60 500 theoretical plates for methyl pentadecanoate.

Reference material

The identifications reported here are built on gas chromatographic retention values and mass spectral fragmentation patterns of natural compounds which have been compared with corresponding data for reference compounds. Most of these data have been obtained by gas chromatography and mass spectrometry of well-characterized compounds in our possession. In a few cases the identifications rely on data obtained from the literature, especially some mass spectra tabulated in *Atlas of Mass Spectral Data* (STENHAGEN *et al.*, 1969).

The capillary gas chromatographic retention values have been measured relative to the nearest hydrocarbon reference compound. A mixture of straight-chain, saturated hydrocarbons C₁₁ to C₂₀ and C₂₂ and C₂₄ has therefore been added to the natural material.

TABLE 4—ANALYSED MATERIAL OF *F. polycetna*, WORKERS

Analysis No.	Date of collection	Locality	Part of the body	No. of ants	Isolation method	Analytical method	Date of analysis
1	28 Aug. 1967	Öl. Skogsby	Whole ants	300	Vacuum degassing	GC/MS	10 Oct. 1967
2	8 Sept. 1969	Öl. Skogsby	Dufour's gland	38	Ether extract	GC/MS	8 Sept. 1969
3	8 Sept. 1969	Öl. Skogsby	Dufour's gland	38	Ether extract	GC	8 Sept. 1969
4	6 Apr. 1971	Göteborg	Dufour's gland	1	Precolumn	CGC	7 Apr. 1971
5	6 Apr. 1971	Göteborg	Dufour's gland	1	Precolumn	CGC	7 Apr. 1971
6	24 Aug. 1971	Öl. Skogsby	Dufour's gland	5	Precolumn	CGC with references	24 Aug. 1971
7	11 Oct. 1971	Öl. Skogsby	Dufour's gland	4	Precolumn	CGC	11 Oct. 1971
8	15 Oct. 1971	Öl. Skogsby	Dufour's gland	1	Precolumn	CGC with references	19 Nov. 1971
9	15 Oct. 1971	Öl. Skogsby	Dufour's gland	2/3	Precolumn	CGC	3 Dec. 1971

GC, Gas chromatography with packed glass columns; CGC, capillary gas chromatography; GC/MS, combined gas chromatography/mass spectrometry.

TABLE 2—VOLATILE COMPOUNDS IN THE SECRETION OF DUFOUR'S GLAND FROM WORKER ANTS OF *F. nigricans*, *F. rufa*, AND *F. polycтена*. THE SUBSTANCES HAVE BEEN ARRANGED IN ORDER OF INCREASING RETENTION VALUES MEASURED RELATIVE TO NORMAL HYDROCARBONS

Compound	No.	<i>F. nigricans</i>	<i>F. rufa</i>	<i>F. polycтена</i>
Nonane	1	×	×	×
3-Methylnonane	2	×		
Decane	3	×	×	×
Undecene	4	×	×	×
Undecane	5	1100	1100	1100
5-Methylundecane	6	1167	1156	(1168)
3-Methylundecane	7	1180	1170	1179
Dodecadiene	8	1190		
Dodecene	9	1195	1192	1194
Dodecane	10	1200	1200	1200
4-Methyldodecane	11	1256		
Tridecene	12	1278	1280	1280
Tridecane	13	1300	1300	1300
5-Methyltridecane	14	1352	1348	(1349)
3-Methyltridecane	15	1369	1368	(1367)
Tetradecene	16	×	×	
Tetradecane	17	1400	1400	(1400)
?	18	1414	×	(1414)
Pentadecadiene	19	1465	1467	(1465)
Pentadecene	20	1474	1476	(1473)
?	21	1487	1488	(1485)
Pentadecane	22	1500	1500	1500
5-Methylpentadecane	23	×		
Hexadecene	24	×		
?	25		1579	1579
Hexadecane	26	1600	1600	(1600)
Tetradecenol	27	1646		1650
Heptadecadiene	28	1663	1663	(1664)
Heptadecene	29	1675	1672	1677
Heptadecane	30	1700	1700	1700
Tetradecenyl acetate	31	×	×	×
Octadecene	32	1769	1767	1772
Tetradecyl acetate	33	×	×	
Octadecane	34	1800	1800	(1800)
Hexadecenol	35	1850		(1842)
Nonadecadiene	36	1863	×	(1858)
Nonadecene	37	1882	1870	1876
Nonadecane	38	1900	1900	1900
Hexadecenyl acetate	39	1968	1964	1968
Eicosene	40	×	1968	1971
Hexadecyl acetate	41	×	1996	(1995)
Eicosane	42	2000	2000	2000
Heneicosene	43	2068	2065	2066
Heneicosane	44	2100	2100	
All- <i>trans</i> -geranylgeraniol	45	2168	2161	2160
Octadecyl acetate	46	×	2196	2195

TABLE 2 (cont.)

Compound	No.	<i>F. nigricans</i>	<i>F. rufa</i>	<i>F. polycтена</i>
Docosane	47	×	×	×
All- <i>trans</i> -geranylgeranyl acetate	48	2274	2267	2267
Tricosene	49			

× Compound identified (through mass spectrum) but retention value not measured.

Number in parentheses relate to compound indicated by retention value only.

Note that retention values lower than 1200 are extrapolated.

A certain difficulty has been encountered in correlating some well-resolved—although small—components in the capillary gas chromatograms with mass spectral data. These have been obtained with packed columns, which have not fully resolved some individual components. A few of the minor components of the secretions therefore remain unidentified.

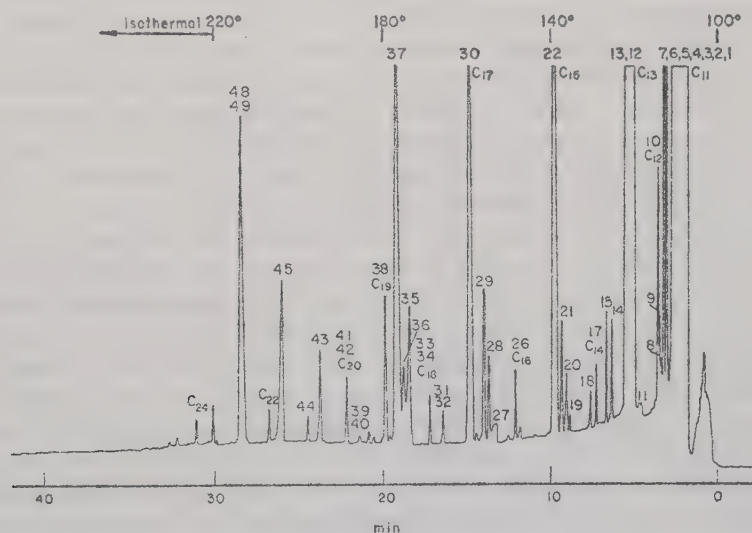


FIG. 2. Capillary gas chromatogram of the volatile material isolated by precolumn technique of three excised Dufour's glands from worker ants of *F. nigricans* (analysis No. 5 in Table 1). The straight-chain saturated hydrocarbons C_{11} to C_{20} , and C_{22} as well as C_{24} have been added as reference compounds. A gas chromatogram without the hydrocarbon reference substances is shown in Fig. 14. The different naturally occurring substances have been numbered according to Table 2. The reference hydrocarbons added have been designated by a C and the number of carbon atoms. Column: 23 m glass capillary coated with Silicone OV-101. Temperature programmed with $4^{\circ}\text{C}/\text{min}$ from 100 to 220°C .

The nomenclature of the substances follows I.U.P.A.C. Organic Nomenclature Rules 1969.

IDENTIFICATION AND STRUCTURE DETERMINATION

F. nigricans, workers

The typical composition of the volatile part of the secretion from Dufour's gland in worker ants of *F. nigricans* is shown by the gas chromatogram in Fig. 2. This emanates from a precolumn run of three glands on a capillary column employing silicone as the stationary phase (analysis No. 5 in Table 1). Note that straight-chain, saturated hydrocarbons C_{11} to C_{20} and C_{22} and C_{24} have been added as reference substances. These are accordingly designated in the figure. In the analysis the precolumn was heated to 135°C for 10 min and the temperature of the separation (capillary) column was programmed from 100 up to 220°C with a rate of 4°C/min. Table 1 summarizes the natural material used in this and other analyses.

Forty-nine of the resolved components in this and the two other species have been numbered and ordered in Table 2, after increasing retention values measured relative to the nearest straight-chain, saturated hydrocarbons. Of these, 46 components have been identified by their mass spectra, in most cases in combination with capillary gas chromatographic retention values. Hitherto unidentified components with known retention values are designated by question marks in Table 2. Components from one of the species which have not been conclusively identified in this species but which have retention values close to components identified in another species have been supposed identical. Absence of mass spectral data are indicated by parentheses in Table 2. The retention values below 1200 have been estimated by extrapolation and are therefore less accurate.

Some of the substances present in trace amounts only have been identified and numbered in Fig. 2 and Table 2. The rest of them are, however, still unidentified and they have not been included in Table 2. This table must not be read as a fingerprint of substances present in the secretion of Dufour's gland in each species. Such a picture can be published only when the annual variation, the nest variation, and perhaps also the individual variation have been investigated. These investigations and biological ones will show if the trace substances are of any importance.

The dominating components in the portion of higher volatility have been identified as straight-chain, saturated hydrocarbons. The whole series of straight-chain, saturated hydrocarbons with from 9 to 22 carbon atoms, undecane being the dominating component, have been found. These correspond to components 1, 3, 5, 10, 13, 17, 22, 26, 30, 34, 38, 42, 44, and 47 in Fig. 2 and/or in Table 2. The uneven members of this homologous series are present in larger amounts than the even ones. These hydrocarbons are accompanied by smaller amounts of monounsaturated, straight-chain hydrocarbons with from 11 to 21 carbon atoms. They correspond to components 4, 9, 12, 16, 20, 24, 29, 32, 37, 40, and

43 in the gas chromatogram. It should be noted that whereas tridecene is the dominating monounsaturated hydrocarbon in the region of the higher volatility, nonadecene is the dominating one in the middle region. Nonadecene is also present in considerably larger amounts than the corresponding saturated hydrocarbon, nonadecane.

Ozonolysis has been performed according to BEROZA and BIERL (1966, 1967) with a sample of 20 excised Dufour's glands of *F. nigricans* (analysis No. 12 in Table 1), extracted with hexane. A mass spectrometric analysis showed that nonanal was the most abundant of the aldehydes produced, followed by decanal. No other aldehydes were found in comparable amounts.

By comparing these results with the proportions of the larger unsaturated compounds, undecene, tridecene, and nonadecene, it is highly probable that they all have their double bonds in position 9, 10.

Doubly unsaturated, straight-chain hydrocarbons have also been identified in trace amounts. Components 8, 19, 28, and 36 are thus identified as dodecadiene, a pentadecadiene, a heptadecadiene, and a nonadecadiene respectively.

We have also found minor amounts of a few methyl branched, saturated hydrocarbons in the region of higher volatility, viz. 3-methylnonane, 5-methyl- and 3-methylundecanes, 4-methyldodecane, 5-methyl- and 3-methyltridecane, and 5-methylpentadecane, corresponding to components 2, 6 and 7, 11, 14 and 15, and 23. These compounds were directly identified by their characteristic mass spectral fragmentation, especially by preferred cleavage of the bond adjacent to the methyl branching. For branching in position 3, 4, and 5 this produces a fragment due to loss of ethyl- (M-29), propyl- (M-43), and butyl- (M-57) respectively. Moreover, four methyl nonanes were used as references. Their retention values were 955, 958, 967, and 960 for 5-, 4-, 3-, and 2-methyl nonane respectively.

Two aliphatic alcohols, tetradecenol and hexadecenol, were found in minor amounts. They correspond to components 27 and 35 and were identified through their mass spectra, augmented by their retention values.

Trace amounts of aliphatic acetates have been identified in *F. nigricans*. Tetradecenyl acetate, component 31, hexadecenyl acetate, 39, and hexadecyl acetate, 41, have been found. As reported below, the amounts of acetates present in this species are much lower than in the other two.

The dominating component in the region of lower volatility of *F. nigricans* has been identified as all-*trans*-geranylgeraniol, component 45. The corresponding acetate, all-*trans*-geranylgeranyl acetate, component 48, is also present, but in small amounts. In the gas chromatogram the acetate is mixed with tricosene. The proportion between components 48 (all-*trans*-geranylgeranyl acetate) and 49 (tricosene) is approximately 1:10 to 1:15. The mass spectrum of natural geranylgeraniol is shown in Fig. 3, and the mass spectrum of synthetic geranylgeraniol is given in Fig. 4. The latter substance was prepared by Professor STINA STÄLLBERG-STENHAGEN (after RUZICKA and FIRMEINICH, 1939). It has also been isolated from linseed oil and characterized by us through comparison with the synthetic product.

Inspection of the mass spectra given in Figs. 3 and 4 show the high degree of resemblance between them. Fragment $m/e = 69$ forms the most abundant peak. It is the characteristic isoprenoid fragment $(C_5H_9)^+$ due to cleavage of the bond between two neighbouring isoprenoid units. The other fragments in the region

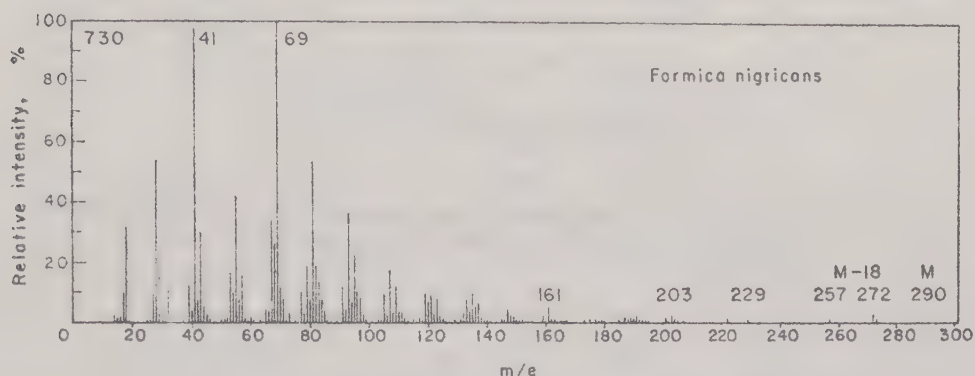


FIG. 3. Mass spectrum of component 45 in the gas chromatogram (Fig. 2) of *F. nigricans*, identified as all-*trans*-geranylgeraniol.

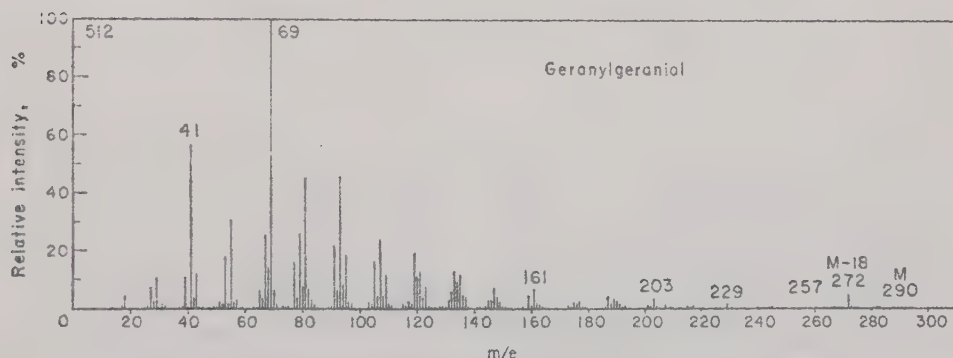


FIG. 4. Mass spectrum of synthetic all-*trans*-geranylgeraniol.

of lower mass are typical for an isoprenoid structure: 81, 93, 107, 119, etc. The most prominent peak in the high mass end corresponds to loss of water $(M-18)^+ = 272$. Loss of water and the basic isoprenoid unit produces a significant fragment $(M-18-69)^+$ at $m/e = 203$. Further loss of isopropyl leads to formation of the characteristic fragment $(M-18-69-43)^+$ at $m/e = 161$. In Fig. 5 the high mass end of geranylgeraniol is shown in relation to fragment $m/e = 161$. The molecular peak $M = 290$ is now resolved. Fragments $m/e = 257$ corresponding to the ion $(M-18-15)^+$ and $m/e = 229$ corresponding to the fragment $(M-18-43)^+$ are also prominent.

The result of admixture of synthetic geranylgeraniol with the volatile secretion from Dufour's gland in *F. nigricans* is shown in Fig. 6. This picture shall be

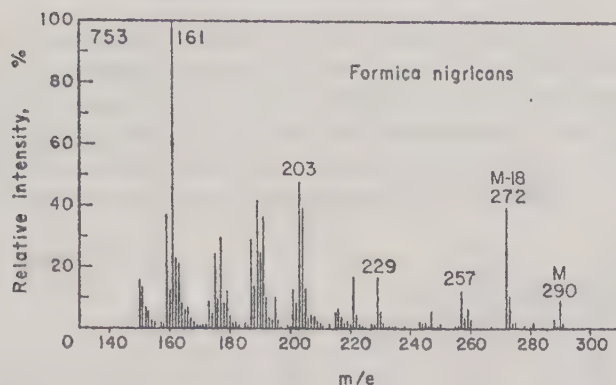


FIG. 5. Mass spectrum of high mass end of component 45 in the gas chromatogram (Fig. 2) of *F. nigriceps*, identified as all-*trans*-geranylgeraniol.

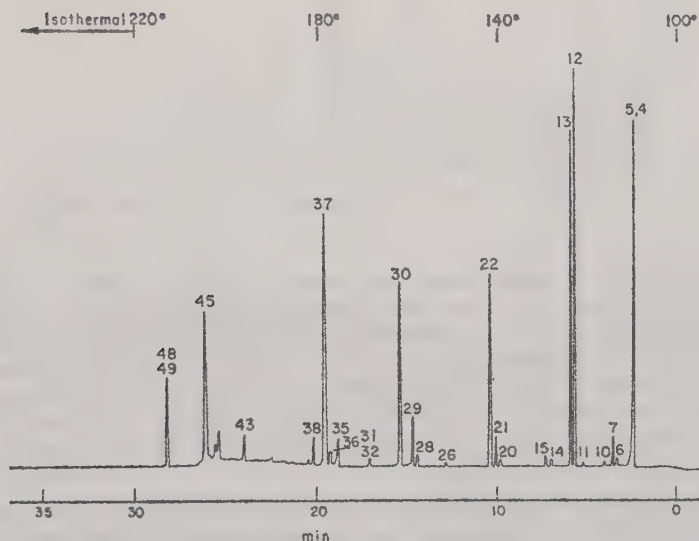


FIG. 6. Capillary gas chromatogram of the volatile material isolated by precolumn technique of one-half of a Dufour's gland from one worker ant of *F. nigriceps* (analysis No. 11 in Table 1). Synthetic all-*trans*-geranylgeraniol (2.5 μ l) has been added to the glandular secretion. Because we were primarily interested in the substances of the low volatile portion of the secretion and in order not to overload the capillary column the natural material was evaporated for some minutes so that the relative proportions of high volatile hydrocarbons were decreased. The gas chromatogram should be compared with that shown below in Fig. 7. The designations of the figure are identical with those in Fig. 2. Column: 28 m glass capillary column coated with Silicone OV-1. Temperature programmed with 4°C/min from 100 up to 220°C.

compared to a gas chromatogram of the natural secretion to which only the straight-chain, saturated reference hydrocarbons have been added, as shown in Fig. 7. If the proportion between components 45 (geranylgeraniol) and 37 (nonadecene) is compared it is obvious that component 45 has increased through the addition. It is also a homogenous peak. This is a further strong indication that component 45 is identical with all-*trans*-geranylgeraniol.

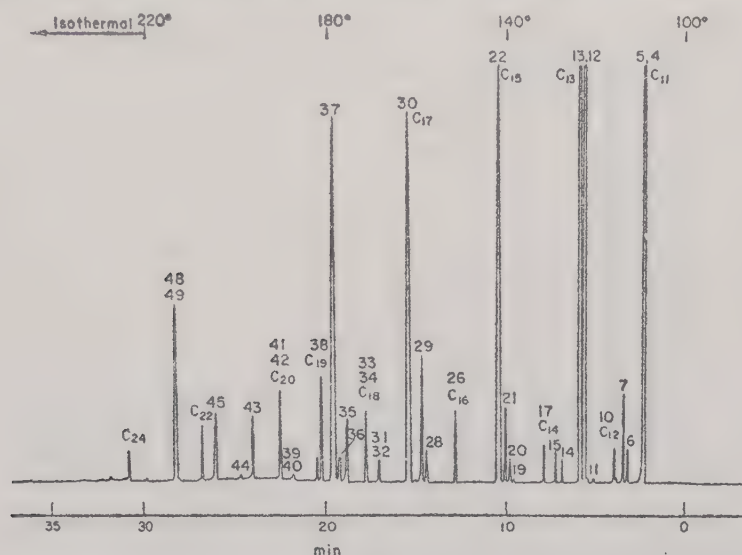


FIG. 7. Capillary gas chromatogram of the volatile material isolated by precolumn technique of Dufour's gland from one worker ant of *F. nigricans* (analysis No. 10 in Table 1). The same hydrocarbon references have been added as in the gas chromatogram shown in Fig. 2, and the designations of the figure are also identical with those in that figure. The high volatile hydrocarbons of the sample have been partly evaporated as for the analysis shown in the gas chromatogram in Fig. 6. The gas chromatographic data are identical with those in Fig. 6.

Component 48, identified as all-*trans*-geranylgeranyl acetate, is mixed with 49, tricosene, in the gas chromatogram in a proportion of about 1 : 10 or less as mentioned above. Both these substances are present also in *F. rufa* and *F. polycтена* but in a proportion of about 1 : 1. The structural evidences for all-*trans*-geranylgeranyl acetate is therefore given below in the report of *F. rufa*.

Between pentadecene, 20, and pentadecane 22, there is in the gas chromatogram in Fig. 1 a minor, still unidentified, component, 21. Two very small peaks are also seen between heptadecene, 29, and heptadecane, 30, and between nonadecene, 37, and nonadecane, 38. In the secretion of Dufour's gland from *Camponotus ligniperda*, which is very similar to the secretion from the three *Formica* species now analysed, we have identified two ketones; 2-tridecanone and 2-pentadecanone (BERGSTRÖM and LÖFQVIST, 1972b). In the gas chromatogram of *C. ligniperda*

the former is observed between pentadecene and pentadecane just as component 21, and the latter between heptadecene and heptadecane. We have therefore carefully checked all mass spectra available in these regions from the three species *F. nigricans*, *F. rufa*, and *F. polycтена* and looked for fragments from ketones, but we were not able to find any. The three small peaks are therefore obviously not ketones.

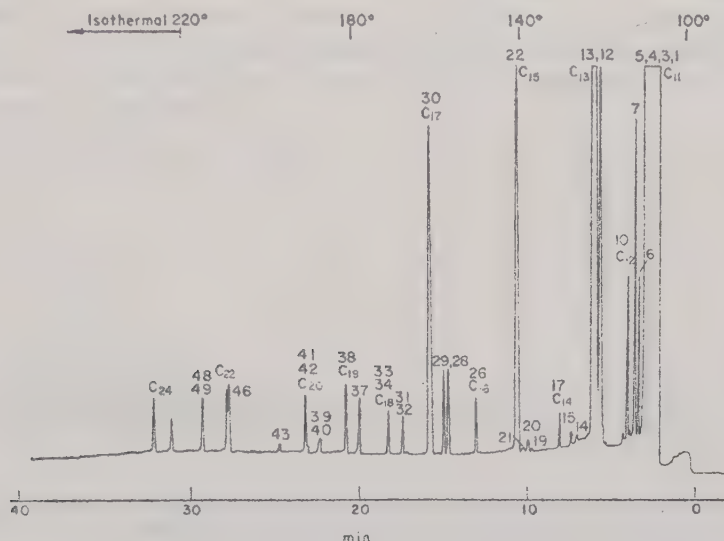


FIG. 8. Capillary gas chromatogram of the volatile material isolated by precolumn technique of Dufour's gland from three worker ants of *F. rufa* (analysis No. 17 in Table 3). The same hydrocarbon references have been added as in the gas chromatogram shown in Fig. 2. The gas chromatographic data and the designations of the figure are also identical with those in Fig. 2. A gas chromatogram without the hydrocarbon reference substances is shown in Fig. 15.

Component 18 present in the secretion in a small amount and a few substances present in trace amounts have not been identified.

The identified compounds have been summarized according to chemical classes in Table 6. Many of the components, particularly the hydrocarbons, evidently constitute homologous series.

F. rufa, workers

The data for analyses of material of this species are summarized in Table 3. Fig. 8 shows a typical capillary gas chromatogram obtained from 3 Dufour's glands with hydrocarbons added as reference compounds. The same numbering of components as for *F. nigricans* has been used (*cf.* Table 2). The composition of the secretion from *F. rufa* shows great similarity with that of *F. nigricans* described above. We prefer to use the adjective 'congruent' for this situation meaning that both the qualitative composition of the complex secretions and the proportions

between individual compounds have a high degree of similarity. The absence in the chromatogram from *F. rufa* of a few of the minor components found in *F. nigricans* might be due to the fact that the glands of the latter species contained more material.

The main difference lies in the composition of the region of lower volatility. Octadecyl acetate is in *F. rufa* a prominent component, No. 46, together with component 48 which has been identified as all-*trans*-geranylgeranyl acetate. On the silicone column this compound has the same retention value as that of tricosene, component 49. The proportion between the two components is, as seen in the mass spectra, approximately 1 : 1.

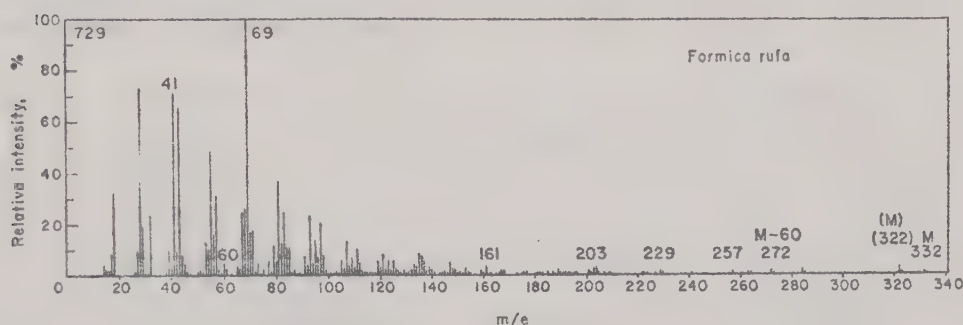


FIG. 9. Mass spectrum of component 48 in the gas chromatogram (Fig. 8) of *F. rufa*, identified as all-*trans*-geranylgeranyl acetate.

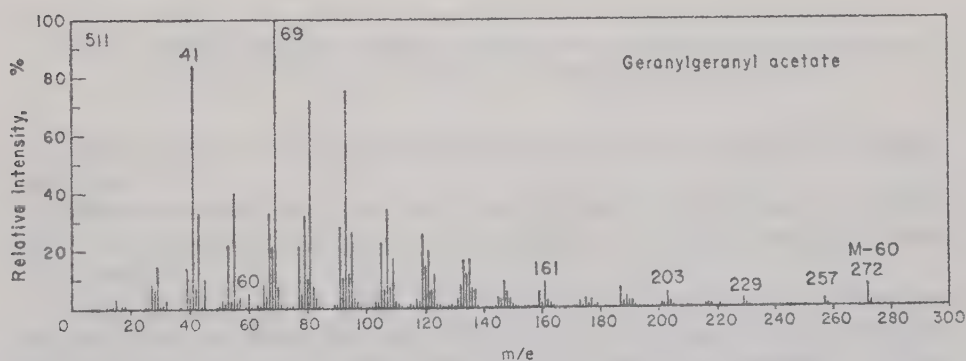


FIG. 10. Mass spectrum of synthetic all-*trans*-geranylgeranyl acetate.

The mass spectrum of natural all-*trans*-geranylgeranyl acetate is shown in Fig. 9, molecular weight $M = 332$. The presence of tricosene is indicated by its parent peak, $M = 322$. As can be seen from the mass spectrum it shows great similarity with that of all-*trans*-geranylgeraniol in Fig. 3. The fragment $m/e = 60$, which corresponds to the ion $(C_2H_4O_2)^+$ is indicative of an acetic acid ester, and fragment $m/e = 272$ which is due to the loss of $(C_2H_4O_2)$ indicates, together with

the characteristic isoprenoid fragments in the lower region, $m/e = 69, 81, 93, 107, 119$, and in the medium region, $m/e = 161, 203$, that the structure is an acyclic diterpene. All-*trans*-geranylgeranyl acetate was prepared from the above-mentioned all-*trans*-geranylgeraniol. The mass spectrum of all-*trans*-geranylgeranyl acetate is shown in Fig. 10, which can be compared with that of the natural component in Fig. 9. They show a high degree of similarity, allowing for the admixture in the latter of tricosene. Even in this case the high mass end is shown separately. This reveals, *cf.* Fig. 11, the molecular ion, $m/e = 332$, as well as fragments $m/e = 272, 257$, and 229 , due to the loss of acetate, acetate + methyl, and acetate + propyl, respectively.

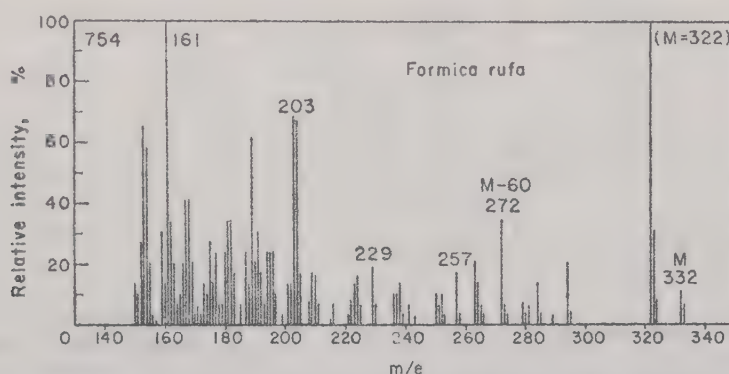


FIG. 11. Mass spectrum of the high mass end of component 48 in the gas chromatogram (Fig. 8) of *F. rufa*, identified as all-*trans*-geranylgeranyl acetate.

The retention value of component 48 also tallies well with that of the synthetic compound.

Some compounds have so far only been detected in *F. nigricans*, *cf.* Table 2, but it must be pointed out that this could be due to the larger material analysed of this species.

We started this investigation on *F. rufa* with an analysis of 300 vacuum degassed whole ants (analysis No. 1 in Table 3). In this analysis we did not find any substances which were not later identified from the secretion of excised Dufour's glands. It is thus evident that this gland is the main source for pheromones in this ant. There must be other pheromones, but these must be present in only minute trace amounts.

F. polycтена, workers

This species was not analysed to the same extent with the gas chromatograph-mass spectrometer and several of the components are therefore indicated by retention values alone, which can be seen from Table 2. A typical capillary gas chromatogram is shown in Fig. 12. Reference hydrocarbons have been added.

This species shows great similarity with *F. rufa*. Just as *F. rufa*, *F. polycтена* also has octadecyl acetate, 46, as a dominating substance together with the two isoprenoids in the high volatile portion of the secretion. In this respect and also in smaller differences in the proportion of the substances discussed below, *F. polycтена* stands closer to *F. rufa* than to *F. nigricans*.

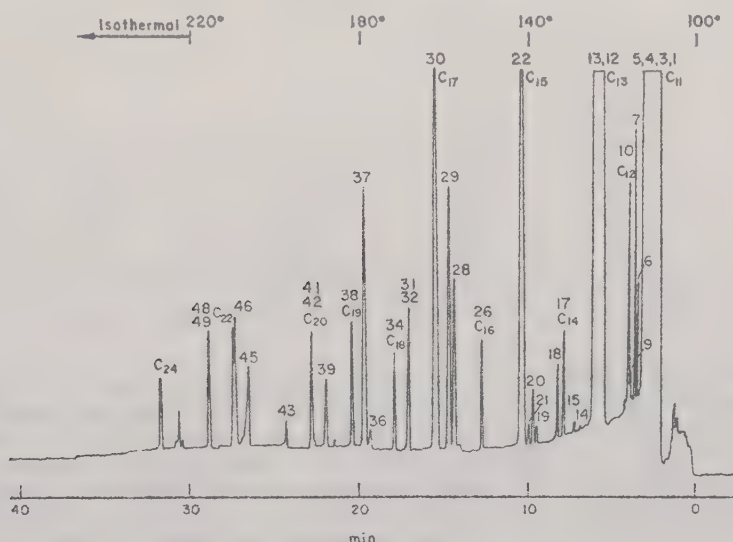


FIG. 12. Capillary gas chromatogram of the volatile material isolated by precolumn technique from Dufour's gland in five worker ants of *F. polycтена* (analysis No. 6 in Table 4). The same hydrocarbon references have been added as in the gas chromatogram shown in Fig. 2. The gas chromatographic data and the designations of the figure are also identical with those in Fig. 2. A gas chromatogram without the hydrocarbon reference substances is shown in Fig. 16.

Also the analysis of this ant began with a sample of 300 vacuum degassed whole ants (analysis No. 1 in Table 4). Just as for *F. rufa* we were not able to find any substance emanating from any other gland than Dufour's gland.

PROPORTION OF THE SUBSTANCES IN THE SECRETION FROM THE THREE SPECIES

The proportions between the major components are shown in Fig. 13 which is a gas chromatogram run on two-thirds of the secretion from Dufour's gland of one ant of *F. polycтена*. We have also calculated the proportions of some of the substances from *F. nigricans*, *F. rufa*, and *F. polycтена*. The figures are shown in Table 5. The figures given for the proportions are only approximate because they are based on only one gas chromatogram from each species. But just as the gas chromatogram in Fig. 13, they show the general picture, with undecane as the main substance followed by longer saturated hydrocarbons. The relative proportions of some of the substances are also discussed below.

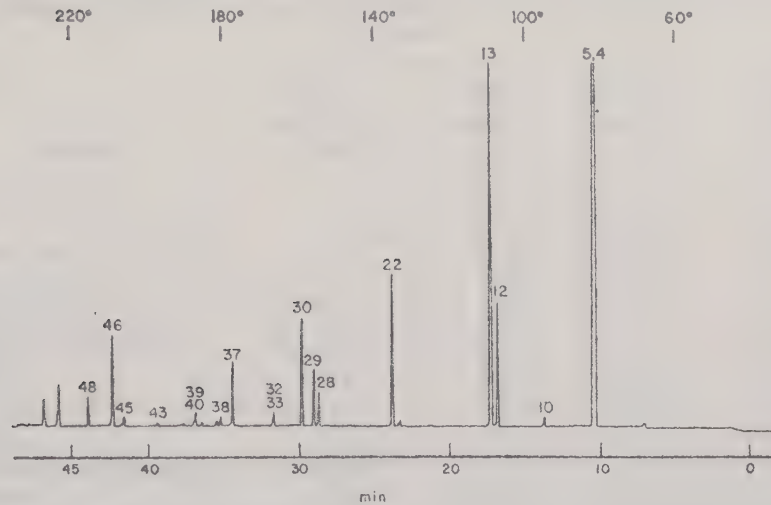


FIG. 13. Capillary gas chromatogram of the volatile material isolated by precolumn technique from two-thirds of Dufour's gland in one worker ant of *F. polystena* (analysis No. 9 in Table 4) showing the relative proportions between the main components. The designations of the substances are identical with those in Fig. 2. Column: 28 m glass capillary column with Silicone OV-1. Liquid nitrogen has been used for subambient cooling of the capillary column when the precolumn was heated. Temperature programmed with 4°C/min from 50 up to 220°C.

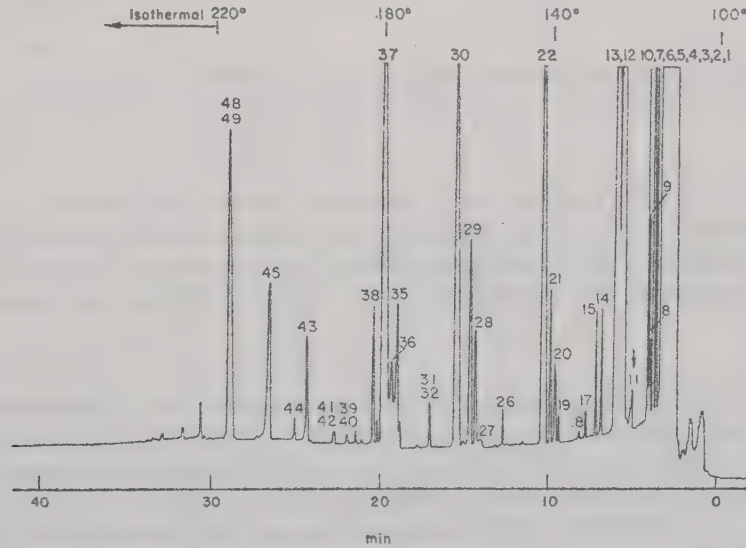


FIG. 14. Capillary gas chromatogram of the volatile material isolated by precolumn technique of five excised Dufour's glands from worker ants of *F. nigricans* (analysis No. 6 in Table 1). The gas chromatogram should be compared with a similar one from *F. rufa* and *F. polystena* shown in Figs. 15 and 16 respectively. The different substances have been numbered according to Table 2. The arrow is explained in the text. The gas chromatographic data are identical with those in Fig. 2.

TABLE 5—THE RELATIVE PROPORTION OF SUBSTANCES PRESENT IN GREAT AND SMALL AMOUNTS IN THE SECRETION OF DUFOUR'S GLAND IN THREE ANALYSES OF *F. nigricans*, *F. rufa*, AND *F. polychena*

Substance	Substance No. according to Table 2	<i>F. nigricans</i> amount in %	<i>F. rufa</i> amount in %	<i>F. polychena</i> amount in %
Nonane	1	—	0, 6	—
Decane	3	0, 6	3, 6	1, 2
Undecene and undecane	4 + 5	56, 3	51, 2	57, 3
5-Methylundecane	6	0, 5	0, 5	0, 1
3-Methylundecane	7	1, 4	1, 6	0, 5
Dodecene and dodecane	9 + 10	0, 5	1, 0	0, 5
Tridecene	12	6, 4	3, 2	2, 9
Tridecane	13	5, 9	19, 9	13, 8
Pentadecene	20	0, 2	0, 2	0, 6
Pentadecane	22	3, 7	5, 6	5, 5
Tetradecenol and heptadecadiene	27 + 28	0, 2	} 2, 0	0, 8
Heptadecene	29	1, 1		2, 9
Heptadecane	30	4, 7	6, 2	5, 4
Octadecane	34	—	0, 1	0, 4
Nonadecene	37	7, 4	0, 9	2, 7
Nonadecane	38	1, 0	0, 3	0, 3
Heneicosene	43	0, 6	0, 2	0, 6
All- <i>trans</i> -geranylgeraniol	45	1, 2	0, 2	0, 6
Octadecyl acetate and docosane	46 + 47	—	1, 5	2, 6
All- <i>trans</i> -geranylgeranyl acetate and tricosene	48 + 49	8, 4	1, 3	1, 4

Note that the proportions have been calculated from only one single analysis of each species: for *F. nigricans* analysis No. 2 in Table 1, for *F. rufa* analysis No. 14 in Table 3, and for *F. polychena* analysis No. 3 in Table 4.

The amounts of the different substances have also been indicated in Table 6 with one, two, and three crosses for trace, small, and large components respectively. It is thus possible to compare the three *Formica* species now analysed with the ten formicine ants previously analysed by us (BERGSTRÖM and LÖFQVIST 1972a, b).

Comparison between the three species

Three capillary gas chromatograms without added hydrocarbons are shown for *F. nigricans*, *F. rufa*, and *F. polychena* respectively in Figs. 14 to 16. The high degree of congruence between the secretion from the three species are evident from the capillary gas chromatograms. Almost the same substances are present in all the species and in about the same relative proportions.

There are, however, small differences between the three species and these are of three kinds: (1) stable quantitative differences in concentrations of compounds which are so strong that they appear to be characteristic, (2) stable differences in

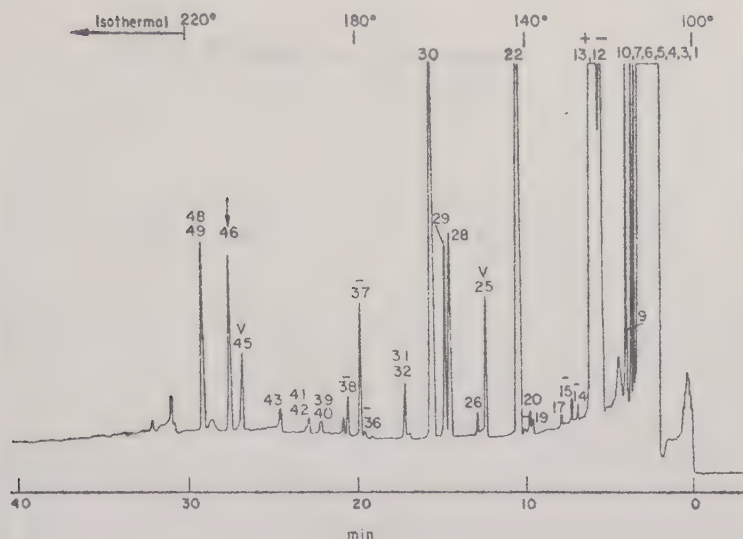


FIG. 15. Capillary gas chromatogram of the volatile material isolated by precolumn technique from five excised Dufour's glands in worker ants of *F. rufa* (analysis No. 18 in Table 3). The different substances have been numbered according to Table 2. Some peaks in the gas chromatogram have been designated with different symbols. These are explained in the text. The gas chromatographic data are identical with those in Fig. 2.

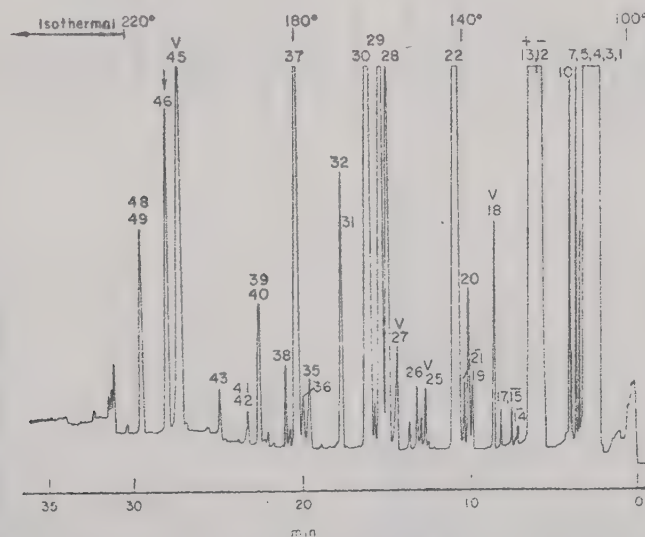


FIG. 16. Capillary gas chromatogram of the volatile material isolated by precolumn technique from four excised Dufour's glands in worker ants of *F. polystena* (analysis No. 7 in Table 4). The different substances have been numbered according to Table 2. Some peaks in the gas chromatogram have been designated with different symbols. These are explained in the text. The gas chromatographic data are identical with those in Fig. 2.

TABLE 6—VOLATILE COMPOUNDS IDENTIFIED FROM THE SECRETION OF DUFOUR'S GLAND FROM WORKER ANTS OF *F. nigricans*, *F. rufa*, AND *F. polycetena*. THE SUBSTANCES HAVE BEEN ARRANGED IN HOMOLOGICAL SERIES

Classes of compounds	Individual compounds	<i>F. nigricans</i>	<i>F. rufa</i>	<i>F. polycetena</i>
Hydrocarbons, straight-chain, saturated  Undecane	Nonane	+	+	+
	Decane	+	+	+
	Undecane	+	+	+
	Dodecane	+	+	+
	Tridecane	+	+	+
	Tetradecane	+	+	+
	Pentadecane	+	+	+
	Hexadecane	+	+	+
	Heptadecane	+	+	+
	Octadecane	+	+	+
	Nonadecane	+	+	+
	Eicosane	+	+	+
	Heneicosane	+	+	+
	Docosane	+	+	+
	Undecene	+	+	+
	Dodecene	+	+	+
Hydrocarbons, straight-chain, monounsaturated  Nonadec-9-ene	Tridecene	+	+	+
	Tetradecene	+	+	+
	Pentadecene	+	+	+
	Hexadecene	+	+	+
	Heptadecene	+	+	+
	Octadecene	+	+	+
	Nonadecene	+	+	+
	Eicosene	+	+	+
	Heneicosene	+	+	+
	Tricosene	+	+	+

the *proportion* of substances, (3) differences in substances fluctuating in quantity so strongly that the differences often or sometimes appear to be qualitative.

In this comparison, *F. nigricans* is first compared to *F. rufa* and *F. polyclena*. The latter two species are finally compared with each other. Differences of the first kind mentioned above have been indicated in the gas chromatograms by an arrow. Thus substances unique for *F. nigricans* are indicated in the gas chromatogram, Fig. 14, of this species. Substances which do not occur in *F. nigricans* but in *F. rufa* and/or *F. polyclena* are indicated in the gas chromatograms of these two species, Figs. 15, 16.

Substances of the second kind are indicated by a plus or minus if their relative proportion in the secretion are higher or lower compared to *F. nigricans*. Substances of the third kind, finally, are indicated by the letter V, which stands for variable.

The comparison is based on all the analyses given in Tables 1, 3, and 4. This is an important point because there are variations in the amounts of different substances in the secretion within the species. We have not in this paper been able to determine if some of these variations are annual or if they are due to experimental conditions. That is an investigation in itself.

For the comparison it is also important to point out that we have for the analyses always used ants from the same nest for each species (except for two analyses of minor importance in *F. polyclena*). When, however, so many factors can vary within a species, a comparison between three species might be considered premature. But if the results are drawn from all the analyses there are smaller differences. Because some of the differences are not so obvious we think it is valuable to point them out as a basis for further study.

The high volatile portion in *F. rufa* and *F. polyclena* is dominated by three substances: geranylgeraniol, 45, octadecyl acetate, 46, and geranylgeranyl acetate, 48. One of these, octadecyl acetate, is completely missing in all analyses of *F. nigricans*. As pointed out above this is the main difference between this species and the other two. It is also in full accordance with our observation that *F. nigricans* generally contains less of the aliphatic acetates.

The absence of octadecyl acetate in *F. nigricans* is a stable and characteristic difference of the first kind mentioned above. There is probably one more of this kind. One small component, 4-methyldodecane, 11, is always present in *F. nigricans* but never in the other two species.

There are also some minor differences in the proportion of the different substances. 5-Methylundecane, 6, and 3-methylundecane, 7, are thus present in relatively smaller amounts both in *F. rufa* and in *F. polyclena* compared to *F. nigricans*. Tridecene, 12, is in *F. rufa* and *F. polyclena* a smaller component than the saturated tridecane, 13. In *F. nigricans* the opposite is found. All these three differences are significant.

Another such substance is the unidentified compound No. 21. It is seen as a minor peak in all capillary gas chromatograms of *F. nigricans* but always in only trace amounts in *F. rufa* and *F. polyclena*.

Approximately equal amounts of heptadecadiene, 28, and heptadecene, 29, are found in *F. rufa*. In the other two species the latter is twice the size of the former. A more apparent difference is that nonadecene, 37, and consequently nonadecane, 38, and nonadecadiene, 36, and probably also hexadecenol, 35, are present in the secretion of *F. rufa* in smaller amounts than in *F. polychteta* and especially in *F. nigricans*.

On the gas chromatographic columns used, geranylgeranyl acetate, 48, does not separate from tricosene, 49. As mentioned above the proportion between these two substances is about 1 : 10 in *F. nigricans* but 1 : 1 in *F. rufa* and *F. polychteta*. This is a stable and one of the largest characteristic differences between the species.

Geranylgeraniol, 45, is an example of a substance present in variable amounts. In the capillary gas chromatogram shown in Fig. 8 of the secretion from *F. rufa* it is thus completely missing. This is abnormal; the ordinary picture in *F. rufa* is that shown in Fig. 15. This is very similar in the relative proportion between geranylgeraniol, 45, octadecyl acetate, 46, and geranylgeranyl acetate, 48, to the capillary gas chromatogram shown in Fig. 12 from *F. polychteta* which shows a typical picture in this respect for that species. The gas chromatogram from *F. polychteta* shown in Fig. 16 is also abnormal. In this gas chromatogram geranylgeraniol can be seen to dominate both over octadecyl acetate and geranylgeranyl acetate. Not only geranylgeraniol but also other alcohols are in this analysis present in unusually high amounts. We do not know why alcohols especially occur in such variable amounts, we can only add that we have noticed it before in *F. sanguinea* (BERGSTRÖM and LÖFQVIST, 1968).

Another substance present in variable amounts is compound No. 18. It is present as a minor peak in both the gas chromatograms from *F. polychteta*, Figs. 12, 16. Note that it occurs in a very high amount relative to tetradecane, 17, in the latter gas chromatogram. The unusually high amount in which alcohols are present in this analysis has been pointed out above. Compound No. 18, is, however, not present in all analyses of *F. polychteta*. In *F. rufa* it is always missing except in analysis No. 15 in Table 3 in which analysis it is present in an amount approximately equal to that of tetradecane, 17. In *F. nigricans* it is always present but only in trace amounts.

There is also another variable substance. It is an unidentified compound No. 25 seen as a rather large peak in Fig. 15 from *F. rufa*. It emerges now and then both in *F. polychteta* and in *F. rufa* but are in other analyses of the two species completely missing.

The alcohol tetradecenol, 27, is finally also very variable in the amount in which it is present in the secretions. It is thus completely missing in the capillary gas chromatograms from *F. rufa*: it is sometimes present in *F. polychteta* and always in *nigricans*. No importance can, however, be attached to these differences because alcohols usually are very variable in their amounts (*cf.*, for example, tetradecenol, 27, in Figs. 12 and 16).

If the differences are summarized it is now evident that there are smaller differences between *F. nigricans* on the one hand and *F. rufa* and *F. polychteta*

on the other. A few of these differences are characteristic but most of them are only differences in the relative proportion of some of the substances.

Between *F. rufa* and *F. polycтена* we have found only one difference significant for all the analyses and it is of minor importance. Nonadecene, 36, and accordingly also nonadecane, 37, and nonadecadiene, 35, are present in the secretion from *F. rufa* in relatively smaller amounts than in *F. polycтена*. To investigate the annual variation within the species and also the variation between different specimens from the same nest and between different nests is a herculian task. But before this has been done the results can hardly be used for any deeper taxonomic or evolutionary evaluations.

DISCUSSION

We have previously analysed the secretion of Dufour's gland from workers of ten formicine ant species (BERGSTRÖM and LÖFQVIST, 1968, 1970, 1972a, b). We have found that the secretion is usually composed of a large number of substances, in most cases of more than 30. Most of these substances are present only in trace amounts. The general picture is that the secretion is built up of two parts. One with a higher volatility in which many of the substances are present in high or relatively high amounts and another part with a low volatility in which the substances mainly are present in trace amounts. Almost all the substances form homological series. The most common are saturated and unsaturated straight-chain hydrocarbons. In some cases there are also aliphatic acetates, in others alcohols and/or ketones or aldehydes. In addition there are usually one or a few isoprenoids. This pattern has been discussed in detail by us in two previous papers (BERGSTRÖM and LÖFQVIST, 1970, 1972a) compared to the results reached by others on formicine ants. We can notice that the results now published from *F. nigricans*, *F. rufa*, and *F. polycтена* are obviously in agreement with the general pattern.

There are several reasons for also identifying substances present in the gland secretions only in trace amounts. The first reason is as a basis for biosynthetic studies.

There have appeared in the literature a few reports which demonstrate through experiments with radioactively marked substances that the insects in these cases themselves synthesize the volatile signals. This is the case for instance with the work on dendrolasin (CASTELLANI and PAVAN, 1966; WALDNER *et al.*, 1969), studies of anisomorphal (MEINWALD *et al.*, 1966), and work of the same group on sesquiterpenes in *Samia cynthia* (HAPP and MEINWALD, 1965). Other studies indicating a *de novo* isoprenoid synthesis by insects include that of CLARK and BLOCH (1969) on the beetle *Dermestes vulpinus* and of GORDON *et al.* (1963) on the green vegetable bug, *Nezara viridula*. Similar studies directed specifically on the biosynthesis of non-terpenoid aliphatic substances acting as odour signals are obviously lacking.

Going further, virtually nothing seems to be known about the exact paths by which these substances are formed. From the composition of the secretions,

plausible routes have been suggested according to the well-established pathways. Thus, FORNEY and MARKOVETZ (1971) have recently discussed the biogenesis of methyl ketones, also in insects. They believe that these compounds are formed either by decarboxylation of β -keto acids, formed during the process of β -oxidation of fatty acids or by oxidation of aliphatic hydrocarbons, and present some evidence for this. Whether unsaturation is achieved through aerobic desaturation or by anaerobic olefin formation as discussed by BLOCH (1969) is also an open question.

The speculation by BURMEISTER and VON GUTTENBERG (1960) that synthesis by isoprenoid route is favoured during anaerobic conditions and that this could account for the seemingly great similarities in biosynthesis between plants and insects is interesting. It links up with the evolutionary viewpoints of BLOCH (1969) and perhaps also with the fact that isoprenoids have often been found to occur together with other aliphatic substances in volatile secretions. We have found this in both the work reported here and in earlier studies. The presence of diterpenes in the *Formica* species analysed now is especially interesting in view of the results by CLAYTON (1964) showing that insects in most cases seem to be incapable of synthesizing steroids from isoprenoid precursors. We have also found diterpenes in the volatile secretions of *Lasius* ants (BERGSTRÖM and LÖFQVIST, 1970), bumblebees of the genera *Bombus* and *Psithyrus* (KULLENBERG *et al.*, 1970), and solitary bees of the genus *Prosopis* (BERGSTRÖM and TENGÖ, in press).

The second reason for identifying the trace substances is as a basis for behavioural studies on the details of the alarm-defence system. It is obvious to every investigator that many kinds of behaviour are shown by single ants and by a group of ants when they are alarmed. Most of these steps or parts in a behaviour situation have been described by WALLIS (1962a, b), WILSON (1962), and more recently and in detail by ROBERTSON (1971). MASCHWITZ (1964) has already shown how essential chemical substances are for ants in their attack on prey or on an enemy. He pointed out that in formicine ants, substances in the poison spray not only alarm other ants of the society but also guide their attack on the victim. ROBERTSON (1971) studied the whole series of behaviour in the alarm-attraction-attack/defence-victim/markings pattern on the ant *Myrmecia gulosa*, an ant from which CAVILL and WILLIAMS (1967) previously have identified a series of hydrocarbons from the secretion of Dufour's gland in workers. Not unexpectedly (reviews by BLUM, 1969; WILSON, 1971) she finds that the substances from Dufour's gland are the most important for releasing all the behaviours mentioned above. But at the same time she points out that the hydrocarbons identified from the gland secretion by CAVILL and WILLIAMS (1967) and present in the secretion as major constituents release a much weaker response than the gland secretion itself. She also states that the reason for this must be substances still unidentified and present in trace amounts in the secretion of Dufour's gland.

One of the most interesting questions is: whether such chemical analyses of gland contents as those on formicine ants now performed can in any way elucidate the systematic of these ants. Some of them such as *Formica rufa* and *F. polycтена* or *Camponotus ligniperda* and *C. herculeanus* are systematically very

closely related. The two forms in each of these species pairs have such weak taxonomic characters that in many cases they can hardly be separated. This is especially evident for *F. rufa* and *F. polychteta*. It is easy to find in the field nests with ants which cannot with certainty be determined to one or the other species.

BUTLER (1967) and many others (reviews by BLUM, 1969; WILSON, 1971) have pointed out that trail pheromones and in still higher degree alarm pheromones in ants are lacking species specificity. As CREWE and BLUM (1970) write, this is really a semantic problem. When KARLSON and LÜSCHER (1959) created the term pheromone only a few such substances were known. In most cases known in those days (*cf.* KARLSON and BUTENANDT, 1959) it was a single substance that released the specific behaviour. Since then a number of investigations besides our own have shown that a secretion from glands with an alarm function in ants is always composed of several or even a large number of substances (among the first were: MCGURK *et al.*, 1966; BERNARDI *et al.*, 1967; CAVILL and WILLIAMS, 1967; BLUM *et al.*, 1968; REGNIER and WILSON, 1968, 1969).

The whole pattern of substances from the secretion of Dufour's gland in worker ants of *F. nigricans*, *F. rufa*, and *F. polychteta* reported here or those from *C. ligniperda* and *C. herculeanus* (BERGSTRÖM and LÖFQVIST, 1972b) can be regarded as species specific. But it is not species specific through the qualitative occurrence in the secretion of the quantitatively dominating substances but rather by the qualitative presence of all substances in trace amounts. Nor is there any reason for the major substances, which release attraction and excitement in alarm behaviour, to be species specific. Of course the alarm behaviour will be exposed almost always within the territory of the home society and probably usually at, on, or in the nest. All the trace substances, on the other hand, may have a species specific importance as recognition marks on enemies in the defence of a nest's inhabitants of their territory.

Considering what has been pointed out above it is not surprising to find that almost every one of 13 ant species now analysed by us concerning the secretion from Dufour's gland usually has the same homologous series of straight-chain saturated hydrocarbons as major components. For the evaluation of the systematic value of the chemical composition of the secretion from Dufour's gland in formicine worker ants the analyses of *F. nigricans*, *F. rufa*, and *F. polychteta* are essential. As pointed out above *F. rufa* and *F. polychteta* are systematically very closely related whereas judging from both morphology and ecology *F. nigricans* stands systematically somewhat farther away from both these species. We now find that also in the trace substances *F. rufa* and *F. polychteta* are almost identical. If we had investigated only these two species the conclusion should have been plausible that they, in reality, are forms of one and the same species. When, however, the composition of the secretion from Dufour's gland is so similar as in the three *Formica* species now analysed the chemical results cannot indicate if *F. rufa* and *F. polychteta* shall be regarded as separate species or not.

The small differences found here between *F. nigricans* and *F. rufa*, which are two valid species, indicate that the differences previously noticed between *C.*

ligniperda and *C. herculeanus* must not be given too great an importance for the evaluation of the systematic position of these two species. Great caution must be shown in judging such cases, especially in applying results from one genus to another. *Lasius niger* and *L. alienus*, for example, are two closely related species which in parts of their range are rather difficult to separate on their taxonomical characters (WILSON, 1955). We have analysed the contents of Dufour's gland from worker ants of these species on Swedish material and found that there are great differences (BERGSTRÖM and LÖFQVIST, 1970). Besides hydrocarbons *L. niger* contains mainly acetates while *L. alienus* contains mainly ketones. Our results from analyses of Dufour's gland in *L. alienus* is in full accordance with those reported by REGNIER and WILSON (1969) from the American form of the same species. But we were not able to discover citronellol or citronellal which REGNIER and WILSON (1969) had identified in secretions from the head of *L. alienus*. This again emphasizes the relatively weak species specificity in the composition of the secretion from Dufour's gland in formicine worker ants.

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Patent No. 1205014

Date of Patent 6 September 1970
Date of Sealing 28 June 1972



Elizabeth the Second by the Grace of God of the United Kingdom of Great Britain and Northern Ireland and of Her other Realms and Territories, Queen, Head of the Commonwealth, Defender of the Faith: To all to whom these presents shall come greeting:

WHEREAS a request for the grant of a patent has been made by

Karl Adolf Lennart Stigmark, Bildsnidarevagen 2, 245 00 Staffanstorp, Sweden, Nils Ingvar Jonsson, Silvas Grand 4, 240 20 Furulund, Sweden, Jan Wilhelm Lofqvist, Winstrupsgatan 7, 222 22 Lund, Sweden, and Hakan Christian Mikael Stenram, Warholms vag 8 B, VIII, 223 65 Lund, Sweden, all Swedish subjects,

for the sole use and advantage of an invention for

Apparatus for automatic indicating of the activity of organisms, especially animals:

AND WHEREAS We, being willing to encourage all inventions which may be for the public good, are graciously pleased to condescend to the request:

KNOW YE, THEREFORE, that We, of our especial grace, certain knowledge, and mere motion do by these presents, for Us, our heirs and successors, give and grant unto the person(s) above named and any successor(s), executor(s), administrator(s) and assign(s) (each and any of whom are hereinafter referred to as the patentee) our especial licence, full power, sole privilege, and authority, that the patentee or any agent or licensee of the patentees and no others, may subject to the conditions and provisions prescribed by any statute or order for the time being in force at all times hereafter during the term of years herein mentioned, make, use, exercise and vend the said invention within our United Kingdom of Great Britain and Northern Ireland, and the Isle of Man, and that the patentee shall have and enjoy the whole profit and advantage from time to time accruing by reason of the said invention during the term of sixteen years from the date hereunder written of these presents: AND to the end that the patentee may have and enjoy the sole use and exercise and the full benefit of the said invention, We do by these presents for Us, our heirs and successors, strictly command all our subjects whatsoever within our United Kingdom of Great Britain and Northern Ireland, and the Isle of Man, that they do not at any time during the continuance of the said term either directly or indirectly make use of or put in practice the said invention, nor in anywise imitate the same, without the written consent, licence or agreement of the patentee, on pain of incurring such penalties as may be justly inflicted on such offenders for their contempt of this our Royal Command, and of being answerable to this patentee according to law for damages thereby occasioned:

PROVIDED ALWAYS that these letters patent shall be revocable on any of the grounds from time to time by law prescribed as grounds for revoking letters patent granted by Us, and the same may be revoked and made void accordingly:

PROVIDED ALSO that nothing herein contained shall prevent the granting of licences in such manner and for such considerations as they may by law be granted: AND lastly, We do by these presents for Us, our heirs and successors, grant unto the patentee that these our letters patent shall be construed in the most beneficial sense for the advantage of the patentee.

IN WITNESS whereof We have caused these our letters to be made patent
as of the eighth day of September
one thousand nine hundred and seventy and to be sealed.



Robert Amis

Comptroller-General of Patents
Designs, and Trade Marks.

PATENT SPECIFICATION

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1265 614

DRAWINGS ATTACHED

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(54) APPARATUS FOR AUTOMATIC INDICATING OF THE ACTIVITY OF ORGANISMS, ESPECIALLY ANIMALS

(71) We, KARL ADOLF LENNART STIGMARK, of Bildsnidarevägen 2, 245 00 Staffanstorp, Sweden, NILS INGVAR JONSSON, of Silvas Gränd 4, 240 20 Furulund, Sweden, 5 JAN WILHELM LÖFQVIST, of Winstrupsgatan 7, 222 22 Lund, Sweden, and HAKAN CHRISTIAN MIKAEL STENRAM, of Warholms väg 8 B, VIII, 223 65 Lund, Sweden, all Swedish subjects, do hereby declare the invention, for which we pray that a patent 10 may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

15 The present invention refers to an apparatus for automatic indication of the activity of organisms, especially animals, e.g. to be used in investigations of the effect of different substances, such as medicines or drugs or 20 different environments, on the activity of animals.

Equipments for investigating the activity of animals are known *per se*. The apparatus generally comprises a space where the 25 animals are kept and an indicating instrument that is affected by the movements of the animals. The apparatus could e.g. consist of a movable plate on which the animals are kept, the movements of the animals implying 30 the plate to activate electric contacts which give rise to electrical signals. The indication will, however, not be very exact and the apparatus could only be used when the number of animals is small. Animals could also 35 be photographed at regular intervals and the film afterwards be studied. The evaluation of the results will, however, in this case be very time-consuming. It is also possible to design an apparatus with an optical or an 40 acoustic indication, the optic apparatus using at least one light beam which affects a photo-cell, the light beam being cut off when the animals move in the space, whereas the acoustic apparatus records the noise derived 45 from the movement of the animals. The essential drawback of the optic apparatus consists in that large areas of the space are

not covered by the light beam, and furthermore, the light could disturb the animals so that the result of the investigation will 50 not be adequate. The acoustic apparatus suffers from an important drawback in that it is very difficult to suppress noise derived from outside the apparatus. Thus, the equipments known *per se*, have very limited applications. 55

In order to eliminate the above mentioned drawbacks and to provide an apparatus which is more generally usable and which gives an adequate indication of the activity of 60 organisms without introducing irrelevant factors in the result, it is an object of the present invention to provide an apparatus which is defined in claim 1.

An embodiment of an apparatus according 65 to the invention will be described in detail below with reference to the enclosed drawings in which Fig. 1 is a block diagram of an apparatus according to the invention, Fig. 2 is a schematical vertical sectional view of 70 the space where the animals are kept, and shows the small capacitor plates arranged in this space. Figs. 3 and 4 are plan views of the bottom or top of the space, according to Fig. 2 and illustrate two different patterns 75 of the capacitor plates, and Fig. 5 is a curve obtained from the registration of the activity of animals in an apparatus in accordance with Fig. 2.

The apparatus according to the invention 80 consists of a transformer bridge 10, which is designed according to principles known *per se*, the bridge comprising two branches formed by the windings of the transformer and two branches, each formed by a capacitor 85 14 and 15 respectively. The bridge is provided with three connections 11, 12 and 13 to which the capacitors 14 and 15 are connected, the connection 12 being connected to the junction of the capacitor branches and to 90 the diagonal of the bridge. The unbalance voltages of the bridge are supplied to a conductor 16, connected to the diagonal of the bridge. If the bridge has been balanced with

predetermined values of the capacitors 14 and 15, i.e. the voltage of the conductor 16 is substantially zero and the capacity of one of the capacitors is changed in relation to the other capacitor due to some outer disturbance this will result in an unbalance voltage at the conductor 16. This fact is used in the apparatus according to the invention for measuring the activity of animals by arranging the capacitors 14 and 15 in a space where the animals are kept, this space being marked with a ring 17 (Fig. 1). Before the different electrical parts connected to the transformer bridge 10 will be discussed, the mechanical design of this space will be described in detail.

The space is shown in Fig. 2 and has the shape of an arena. This arena consists of a cylindrical tube 18, e.g. made by transparent polymethacrylate-glass and is provided with a bottom 19 and a top 20, which can be slid in the tube and which is made in the same material as the cylinder 18. The bottom as well as the top are axially penetrated by a number of metal threads 21 which end in a head 22 at the upper surface of the bottom and the lower surface of the top respectively. These heads form the above-mentioned small capacitor plates and are connected to each other under the bottom and over the top respectively via connections 23 according to a certain pattern, the heads being arranged in triads, one head in each triad being connected to the connection 12 and thus forming the capacitor plate common to the capacitors 14 and 15, whereas the other two heads in each triad are connected to the connection 11 and the connection 13 respectively and form the other capacitor plate of the capacitors 14 and 15. Figs. 3 and 4 show two different ways in which the heads could be connected to each other. In these figures the heads which are connected to each other and to the connection 12 are indicated by a square, the heads connected to each other and to the connection 11 are indicated by a triangle and the heads connected to each other and to the connection 13 are indicated by a circle. It is obvious from the figures that in Fig. 3 the heads are arranged in a triangular system, whereas in Fig. 4 the heads are arranged in a square system. Both systems are equally usable. The distance between the heads is suitably somewhat larger than the length of the animals to be investigated. Due to the fact that the bottom and the top are relatively thick the connection 23 will be situated at a rather large distance from the head 22 which means that the connection does not have to be shielded in order to obtain a capacitive isolation of the head. Such a shielding is, however, within the scope of the invention. The heads 22 are covered by a plastic layer 24 and between the bottom and the top a cylindric cage 25 in which the animals are

kept is inserted in the arena. The cage could be made from a net or consist of a foraminous transparent box. It will thus be possible to keep the animals in these cages outside the arena before the investigation is carried out in order that the animals will be accustomed to the environment of the experiment. The disturbance of the animals will also be considerably smaller if they are kept in a cage when they are moved to the arena than if they are picked into the arena when the experiment is to be carried out. In the bottom 19 (or in the top 20) a channel 26 is arranged. Through this channel the cage is connected to the environment, which makes it possible to change the atmosphere or other characteristics in the cage 25. Since the arena and the cage are transparent, it is also possible to change the light conditions and to watch the animals so that the results of the experiment could be compared with the visually registered activity of the animals.

The transformer bridge 10 is connected to an oscillator 27, the frequency of which has such a value that the animals kept in the cage 25 are affected as little as possible by the electric field derived from the capacitors 14 and 15. A suitable frequency is 15 kHz. When the animals kept in the cage 25 move the capacity of the capacitors 14 and 15 is changed because of the variations of the dielectric constant of the capacitors, and if the transformer bridge 10 was balanced when the experiment started, that is the voltage at the conductor 16 was substantially zero, each disturbance of the balance conditions will give rise to signals from the bridge via the conductor 16. This conductor is connected to a preamplifier 29 in which the obtained signals are amplified. The amplified signals are transferred to a phase-sensitive detector 30 which is controlled by the oscillator 27 which is indicated by the conductor 31. The animals in the cage 25 might give rise to smaller or bigger constant unbalances in the bridge, but since it is not the purpose of the apparatus to register these constant unbalance voltages, the signal derived from the phase-sensitive detector 30 is supplied to a differentiating RC-network 32 at the output of which only the variations of the voltage obtained from the detector 30 will appear. The time constant of this RC-network should be small in comparison with the shortest possible interval between the changes of the unbalance voltage. When the animals in the cage 25 move, pulses will thus be obtained from the RC-network 32, each pulse being derived from a change of the unbalance conditions and these pulses indicate the activity of the animals. An indicator 33 is connected to the RC-network. The indicator could either be designed to indicate each pulse or to integrate the pulses during a certain period and indicate the sum of pulses during this period. The indicator

might also be designed so as to produce a diagram of these sum values. In this case the indicator is provided with a plotter, from which a diagram of the total number of pulses during predetermined intervals is indicated as a function of time. In this way a diagram that indicates the activity of the animals as a function of time is obtained. Such a diagram is shown in Fig. 5. The diagram is obtained from animals which temporarily have been stimulated to a bigger activity which has given rise to a peak in the curve. Since it is possible to watch the animals when the curve is plotted, it is also possible to evaluate the graphically obtained result which could be related to the watched activity. It is also possible by carrying out a series of experiments to show that the registered activity increases proportionally to the number of animals.

The above described apparatus implies several advantages which have been pointed out above and which makes the apparatus well suited for the investigation of animals of different kinds. The most important advantage consists of course therein, that a reliable and easily interpretable automatic registering can be provided. Another important advantage consists therein that it is possible to introduce animals into the arena without disturbing them and to provide variations of the environment by e.g. changing the atmosphere, the light intensity, etc. The invention is, however, by no means limited to the embodiment shown above but could be modified within the scope of the enclosed claims in order to suit different purposes of investigation and different animals.

It is suitable to make the capacities of the capacitors 14 and 15 substantially equal when the arena is empty but it is also possible to use only one of these capacitors and to have the other capacitor arranged outside the arena. This capacitor could then be adjustable or have a constant value. From the detector 30 a certain direct voltage level is obtained. This level could represent either the capacitive or the resistive component of the signal obtained from the amplifier 29. The indicating device that is used should have a resolution that is sufficient compared with the activity of the animals in order to give a detailed result of the activity of the animals. It should also be mentioned that the arena when used for bigger animals, such as rabbits, guinea-pigs or mice might comprise only the bottom 19 or the top 20 and the capacitor heads 22. Thus a net cage in which the animals are kept, might be combined either with the bottom or the top and the design of the arena will then be very simple. The arena shown in the figure has been designed

primarily to be used at investigations of small animals such as insects.

WHAT WE CLAIM IS:—

1. Apparatus for automatic indication of the activity of organisms, especially animals, comprising a space in which the organisms are kept and an indicating device which is affected by the movements of the organisms, characterized in that at least one of the walls of the space is provided with a plurality of capacitor plates which are small in relation to said at least one of the walls, said plate forming at least one capacitive branch in a transformer bridge, the diagonal of the bridge being connected to a circuit sensitive to changes of the output of the bridge, said circuit generating pulses when the bridge output changes and being connected to the indicating device.
2. Apparatus according to claim 1, characterized in that the capacitor plates consist of heads arranged at the end of supporting and connection means.
3. Apparatus according to claim 2, characterized in that the ends of the supporting and connection means remote to the heads are connected to each other in accordance with a predetermined pattern, some of the heads thus forming one plate of the capacitor, whereas the others form the other plate of the capacitor.
4. Apparatus according to claim 2 or 3, characterized in that the heads are arranged on one side of the wall, whereas the connections are arranged on the opposite side of the wall.
5. Apparatus according to claim 2, 3 or 4, characterized in that the space comprises two opposite walls provided with said heads.
6. Apparatus according to claim 5, characterized in that one of said walls is slidably movable in relation to the other wall.
7. Apparatus according to claim 6, characterized in that a separate cage is arranged between the walls.
8. Apparatus according to any one of the preceding claims, characterised in that said circuit comprises a phase-sensitive detector connected to the diagonal of the bridge and an RC network connected to the output of said detector.
9. Apparatus for automatic indication of the activity of organisms, substantially as hereinbefore described with reference to and as illustrated in the accompanying drawings.

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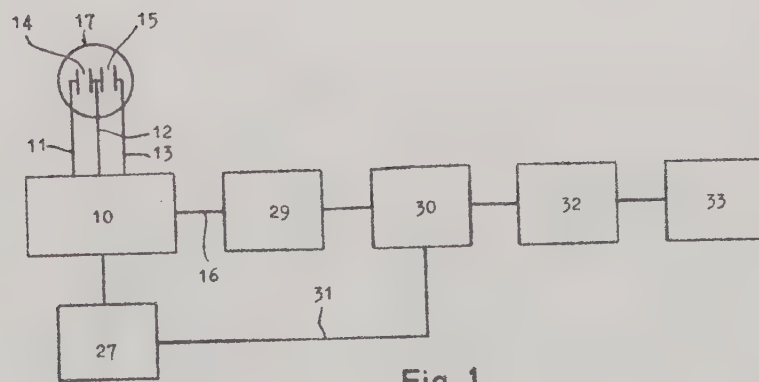


Fig. 1

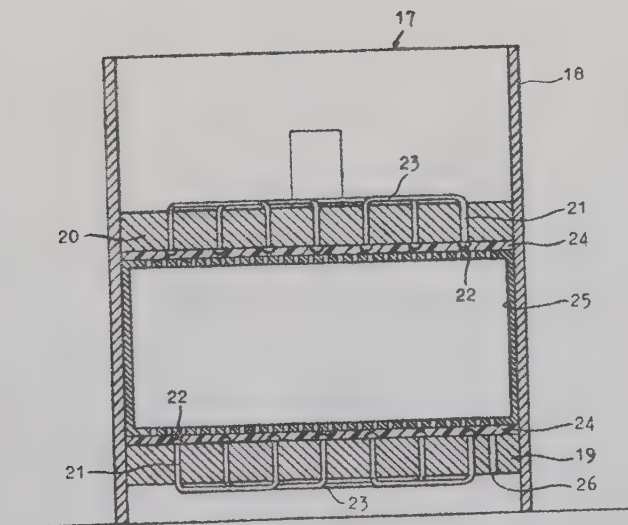


Fig. 2

1265614 COMPLETE SPECIFICATION

2 SHEETS

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the Original on a reduced scale*
Sheet 2

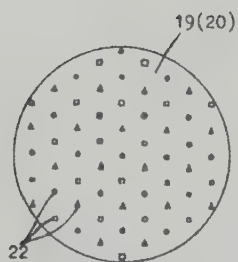


Fig. 3

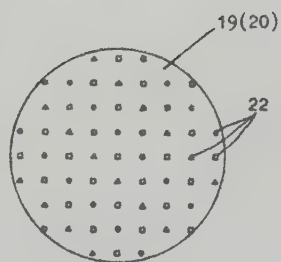


Fig. 4

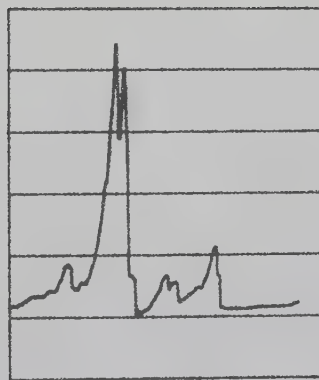


Fig. 5

FORMIC ACID AND SATURATED HYDROCARBONS AS ALARM PHEROMONES FOR THE ANT

FORMICA RUFA L.

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Abstract - Releasers for the most intense, the fast-running, phase of the alarm behaviour were studied in worker ants of the formicine ant Formica rufa L. The investigation was performed with a new technique in which the intensity and the duration of the behaviour pattern were measured in an objective and automatic way.

Workers of formicine ants eject a mixture of formic acid and the secretion from Dufour's gland against an enemy. The secretion from this gland in F. rufa consists of a great number of substances, 41 of which have been identified. The dominating substances form a homologous series of aliphatic, saturated hydrocarbons. Some of these hydrocarbons as well as the formic acid are alarm pheromones. The behavioural threshold value for one of these compounds, decane, was lower than $5 \cdot 10^{13}$ molecules cm^{-3} air. The threshold value for formic acid was estimated to $7 \cdot 10^{15}$ molecules cm^{-3} air. Formic acid and undecane are shown to belong to different reaction groups. The hydrocarbons, on the other hand, seem to affect the same kind of acceptor or receptor.

The total intensity and duration of the alarm behaviour released by formic acid and one hydrocarbon are additive if the two substances are combined in a stimulus. A combination of two hydrocarbons and formic acid release a stronger behaviour than one hydrocarbon with formic acid even when the two stimuli contain the same number of molecules. The hydrocarbons have a combined effect and their relative concentrations regulate the intensity and duration of the alarm behaviour.

INTRODUCTION

The chemical composition of pheromone secretions in formicine ants has been studied in detail for some species (BERGSTRÖM and LÖFQVIST 1968, 1970, 1972 a, 1972 b, 1973, BROPHY et al. 1973, HUWYLER et al. 1975, REGNIER and WILSON 1968, 1969). The secretion has always been found to be a mixture of several substances. Forty-one substances have been identified from a gland secretion in worker ants of Formica rufa (BERGSTRÖM and LÖFQVIST 1973). Twelve of these substances were present in amounts exceeding 1 % of the total secretion (cf. Table 1) : the rest were found as traces only.

There are at least three possible explanations for the complexity of a pheromone secretion:

1. A specific behaviour is released by a single substance only. The other substances are either releaser signals for other behaviour patterns or they are by-products from the biosynthesis.
2. Some of the substances in combination release a specific behaviour. The rest of the substances are signals in another behaviour pattern or by-products.
3. All the substances act together and release a specific behaviour.

The fact that so many substances are present in secretion from ants in an amount exceeding 1 % and the fact that many of them form homologous chemical series indicate that several substances act together as releasers of a specific behaviour in these animals.

The substances identified from workers of F. rufa emanate from the secretion of Dufour's gland (BERGSTRÖM and LÖFQVIST 1973). This gland opens in the short duct of the poison vesicle in the tip of the gaster (FOREL 1878, ROBERTSON 1968). It is probable that the contents of the gland are released together with formic acid from the poison vesicle, as has been shown for the formicine ant species Acanthomyops claviger (REGNIER and WILSON 1968).

The contents from Dufour's gland in formicine ants act as signals in their alarm-defence behaviour (MASCHWITZ 1964). Single, chemically-identified releasers of the alarm behaviour have been studied for several species (REGNIER and WILSON 1968, 1969; BERGSTRÖM and LÖFQVIST 1970, 1972 a).

The biological role of complex secretions is, however, unknown. The aim of the present paper is to investigate single substances as releasers of alarm behaviour in workers of F. rufa and their role in combination with one or more substances.

MATERIALS

The ants were collected from two separate nests at Sjöbo, Skåne, the southernmost part of Sweden. Taxonomically, the ants were typical Formica rufa L. (LANGE 1958, BETREM 1960).

About 1000 ants were collected each week from the surface of the nest together with nest material. After a few hours the ants were transferred to two ventilated perspex boxes, each with a volume of about 45 dm³. The ants were fed sugar and water. The humidity inside the boxes was above 90 % r.H. ; the temperature on the nest surface about 25°C and in the nest bottom about 18°C ; the light intensity 30 000 lux from a halogen bulb and the day length 16 hours from 05 00 h.

Ants collected at the end of one week were used in experiments in the following week. Ants used in experiments one day were put back into the perspex boxes in the evening but not until the ants for the next day's experiments had been collected.

The formic acid (E.Merck, Darmstadt) used had a purity of min. 98 %. The purity of the hydrocarbons (FLUKA AG) was "purissimum", i.e. >99 %.

METHODS

Measuring instruments

For an experiment a box with 20 ants is placed between two perspex discs, one below and the other above the box. In each perspex disc there are 58 sensors, which are parts of a differential condenser (STIGMARK et al. 1972). The sensors are uniformly distributed over the discs with a central distance between the sensors of 20 mm. This part of the instrument is called the arena. The capacity of the condenser is influenced by animals moving over the sensors. The capacity variations are used as a measure of the locomotor activity of the animals and measured by a capacity detector (STIGMARK et al. op.cit.).

The signals from this are fed to an analyser. These two instruments give an almost absolute correlation between the locomotor activity of the animals and the figures registered for their locomotor activity.

The capacity variations are transformed to pulses, which are counted by an electronic counter (Ortec Inc. Mod. 772) and printed out (Ortec Inc. Mod. 777) after intervals determined by an electronic timer (Ortec Inc. Mod. 719). The printing out of the data makes it possible to handle the material statistically. An analog signal is at the same time fed to a pen recorder (Goerz Electro Mod. RE 541).

Presentation of the test substances to the ants

Substances were introduced to the ants as a few cubic centimeters of saturated gas because the gas phase is the natural mode of stimulation for the ants. The approximate number of molecules is known in such a stimulus. A test substance were evaporated to saturation from a small glass vessel inside a plastic syringe (KAFKA 1970). Two syringe sizes were used, 5 and 20 cm³. Each substance was injected from the syringe into the measuring box through a small teflon tube with a length of 50 mm and a diameter of 1.2 mm. The injection has been performed with an automatic injector with a constant speed of 1 cm³ sec⁻¹. One cm³ was the minimum gas volume tested. The inner volume of the plastic tube and steel cannula is 0.066 cm³. This gives a maximum error of 6.6 %. In most experiments, however, a test volume of 4 or 8 cm³ was used, which gave a lower error.

Performance of the experiments

The experiments were carried out at the following conditions: temperature + 23.0 ± 0.3°C ; R.H. 70 ± 4 % ; light intensity 30 000 lux, daylight spectrum ; daylength 16 hr from 05.00 h.

Twenty worker ants of about the same size were used in each experiment. The ants were carefully picked up and after being anaesthsized with carbon dioxide transferred to a perspex box (120 mm in diameter, 20 mm high) with floor and roof of nylon net. The ants were kept during the night in the test boxes at an r.H. > 95 %. They were fed with water and conditioned to the constant temperature of the laboratory. The next day the ants were conditioned for 10 min. to the rela-

tive humidity of the laboratory before the experiment. By this routine the chemoreceptors of the ants recover if - as sometimes happens when handled - some ants have given off some formic acid.

The test boxes were washed in ethanol after each experiment.

Two independent instruments, each with its own units of registration, were used. The two instruments were calibrated against each other to the same sensitivity, and so that only the locomotor activity of the ants was registered but not cleaning and other small movements.

The experiments were carried out in the following way. The spontaneous locomotor activity of the ants was measured for at least 10 min. Then the contents of a control syringe with pure air were injected into the test box. In most cases the ants did not react at all to the air stream (cf. Fig. 1). In some cases, however, the ants respond with a decreasing spontaneous locomotor activity for shorter or longer time (cf. Fig. 4 a-f).

The ants were stimulated with the test substance a few minutes, in later experiments always five, after the control stimulation. The test substance usually released an intense alarm behaviour of the ants. The intensity and duration of this behaviour were measured to a point after the stimulation when the locomotor activity of the ants showed a minimum. Usually more than 5 min. after the alarm behaviour reaction was regarded to have faded out fresh air was flushed through the test box. The air passed through 58 ventilation holes (each with a diameter of 1.6 mm) uniformly distributed over the two perspex discs with the sensors. The air ventilation stream was $1 \text{ m}^3 \text{ min}^{-1}$. This figure should be compared to the volume of the test box, which was 226 cm^3 . The arena was ventilated for at least 10 minutes between each experiment.

The intensity of the locomotor activity of the ants during the experiments was measured, counted and printed out at time intervals (cf. above), mostly one minute. Just before the stimulation with a test substance the time interval was changed from 1 min. to 6 sec., so that the intensity of the alarm reaction could be determined with a greater exactitude.

ALARM BEHAVIOUR STEPS

The following steps of alarm behaviour could be distinguished:

1. Ants walking randomly stop moving, raise their antennae and wave them around in the air.
2. The mandibles are opened. The antennae are still in the positions described.
3. The ants walk slowly towards the odour source with opened mandibles and raised antennae.
4. If the alarm stimulus is strong the ants begin to move very fast. They quickly move around each other looking for an enemy. This is "the fast-running phase" of the alarm behaviour.
5. If an ant meets an intruder during the fast-running phase it immediately attacks. An attack involves a long series of behavioural patterns, which will not be dealt with in this paper.
6. Cleaning of the antennae and the abdominal tip.

A very weak alarm stimulus releases only response No.1. A slightly stronger stimulus is also answered with behavioural patterns Nos. 2 and 3. A strong alarm stimulus is answered by behaviour No. 4. In such cases the reaction time is so short that it is impossible to discern steps Nos 1-3 separately. After a short time in the fast-running phase without any enemy being found this behaviour fades out. Only an enemy releases the attack behaviour sequence. The behavioural chain is interrupted.

After the alarm behaviour such as occurs in response to an attack the ants begin cleaning their antennae and their abdominal tip. They do this even if they themselves have not secreted any formic acid or other substances from their abdominal tip glands. This behaviour is apparently a fixed part of their behaviour after strong alarm-attack reactions.

The alarm behaviour of F. rufa worker ants is very similar to that described by REGNIER and WILSON (1968) for another formicine ant, Acanthomyops claviger. Parts of the behaviour have also been described by WALLIS (1962 a) for Formica fusca. The aggressive behavioural steps have been described by WALLIS (1962 a and b) for F. fusca, and in detail by ROBERTSON (1971) for Myrmecia gulosa.

MEASURED AND CALCULATED VARIABLES OF THE ALARM BEHAVIOUR

Total intensity of the alarm behaviour

It is possible using a different arrangement of the sensors to measure the movements of a single ant in detail. Ants are however social, so I have chosen to measure the alarm behaviour from a group of 20 ants. The locomotor activity of an ant is registered as a pulse each time it passes over a sensor. The locomotor activity of the ants is not measured when they move outside the sensors. Because the sensors are distributed in a regular pattern over the floor and roof in the test box there is, however, a fixed probability for a walking ant to pass over a sensor. This probability is independent of the walking speed of the animals. The number of pulses registered over a period is thus the value estimated with a fixed probability of the summed length of the distance walked by the ants in that period. The value is called the total intensity of the alarm behaviour. It has been estimated for a time period called duration of alarm behaviour (cf. below). The value for the intensity of the alarm behaviour is based almost only on step No. 4 (cf. above), the fast-running phase, in the alarm behavioural chain.

Duration of the alarm behaviour

The fast-running phase of the ants' behaviour is released almost immediately when the ants are stimulated with an alarm pheromone (cf. Figs. 1 and 4 a - f). The only exception is when the alarm stimulus is very weak. It is therefore easy to determine where the fast-running behavioural phase starts. It is a little more difficult to decide when it ceases. Generally, this phase was considered to have ended when the locomotor activity showed a clear minimum for a period of 6 sec. Such a minimum usually occurs when the ants start to clean themselves. A cleaning ant does not show any locomotor activity. After cleaning the ants begin to move again in a kind of spontaneous locomotor activity. Since the ants do not leave the fast-running phase and start cleaning at the same time the spontaneous locomotor activity of some ants may conceal the minimum locomotor activity of those ants that are still stimulated. The end of the fast-running phase has therefore been calculated in the following way. A 6-sec mean value of the spontaneous locomotor activity has been calculated for a period of usually

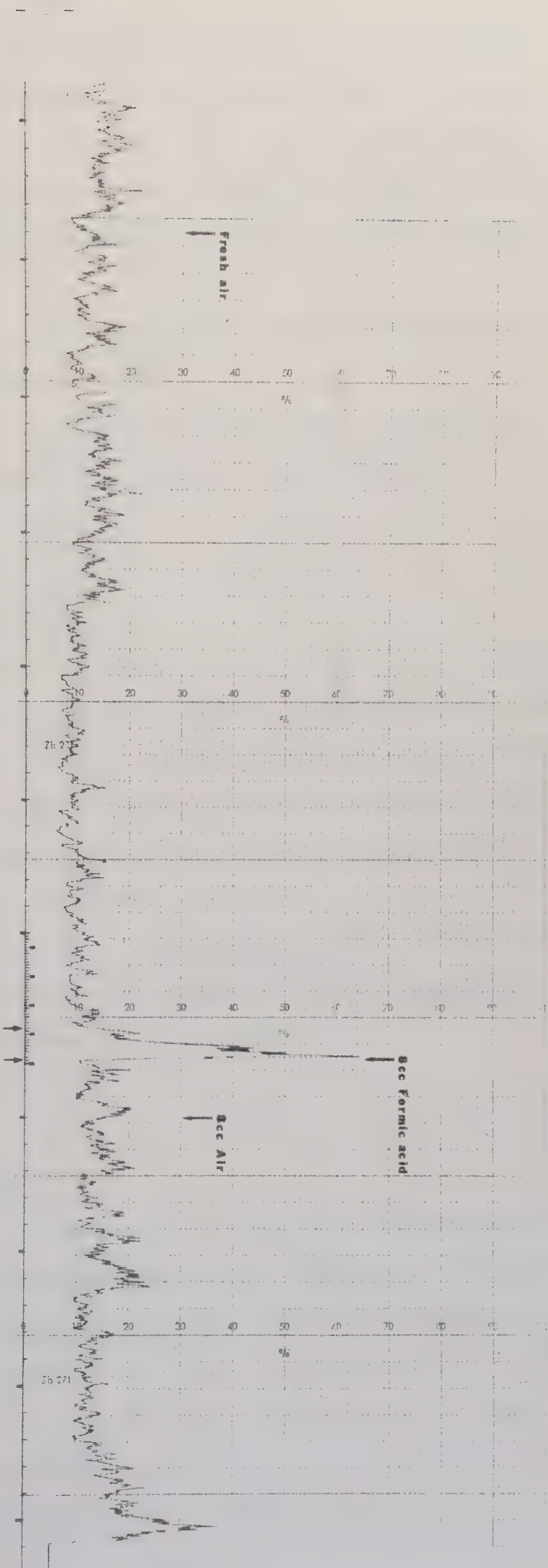


FIG. 1. Kym recorder diagram of the locomotor activity of 20 worker ants of F. rufa. The ants have been stimulated with 8 cm³ saturated gas of formic acid, which releases an outburst of high locomotor activity indicated as a high peak in the diagram. The peak shows the intensity and duration of the fast-running phase of the alarm behaviour released by the formic acid. The beginning of the stimulation is indicated with an arrow below the time scale as is also the end of the fast-running phase of the alarm behaviour. One-minute intervals are shown by the indicator strokes above the time scale except during stimulation when it is divided into 6-second periods. Five-minute strokes are indicated below the time scale, and above the 6-second intervals one-minute intervals have been indicated. The time scale is not the base line for the locomotor activity curve. The base for this is shown by its initial horizontal line. The diagram should be read from the right to the left.

5 min before the ventilation of the test box with fresh air. This is the mean intensity of the spontaneous locomotor activity of the ants after the experiments. To this value have been added 10 pulses. The first 6-sec value after the maximal value of the fast-running phase, which was equal to or lower than the calculated value has been regarded as minimum and the end of the fast-running phase.

The duration of the alarm behaviour is calculated from the beginning of the stimulation until the terminal value.

Because the pen recorder curve is built up of short pulses, collected on a condenser system, there is an inertia in the system. A short minimum is unfortunately not clearly seen.

Maximum intensity of the alarm behaviour

The maximum intensity of the fast-running phase can be very high for a short while, or moderately and uniformly high for a slightly longer period. The 6-sec interval with the highest number of pulses has been used to indicate the maximum intensity of the alarm behaviour. This is not the true maximum because of the fixed limits of the 6-sec intervals. The registration method does not permit, however, any better estimation of the maximum intensity.

The reaction time from the beginning of the stimulation and up to the onset of the response or to the maximum intensity is shorter than the registration period of 6 seconds, and has therefore not been estimated.

RESULTS

Formic acid as an alarm pheromone

It is obvious from Fig. 1 that formic acid is a very strong alarm pheromone for workers of F. rufa. The ants respond immediately to formic acid with an intense fast-running behaviour. The maximum intensity of the alarm behaviour is rather high and the duration short. In Fig. 1 the fast-running phase of the alarm behaviour fades out completely after 66 sec. Such a short duration is typical for formic acid. With the concentrations used (cf. Fig. 2) the intense part of the alarm behaviour has a duration of only about 1 min.

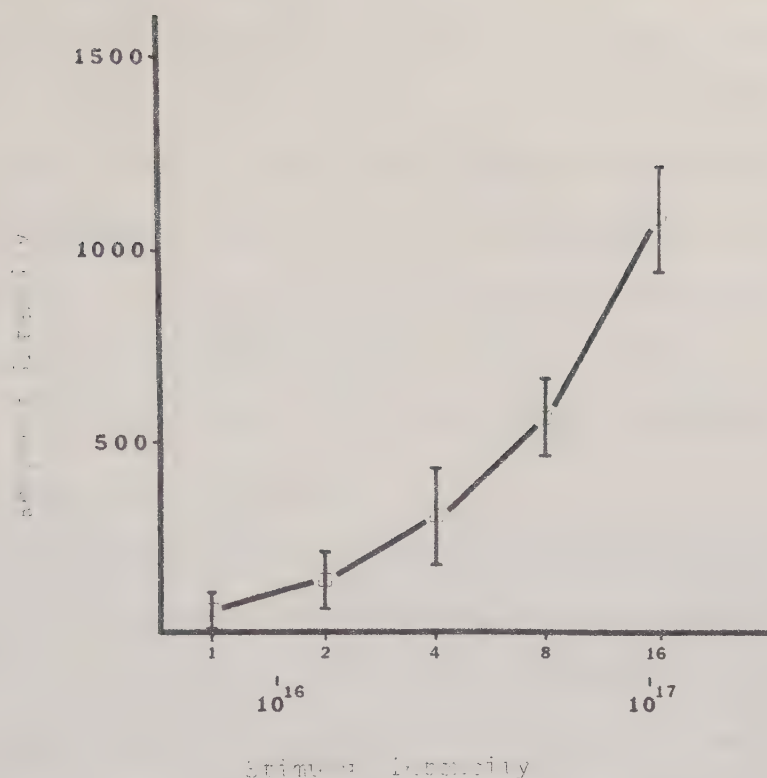


Fig. 1, response curve shows as the total intensity of the fast-running phase of the behavioural response of *20* worker ants of *F. rufa* stimulated with 1-16 cm saturated sol. of formic acid. The standard deviation is shown for each value, which in this series is a very value for four experiments.

The ordinate shows the intensity of the behavioural step in number of pulses min^{-1} registered, and the abscissa the stimulus amount in cm³ of formic acid at a saturated sol. The figures denoting the number of molecules of formic acid cm⁻³ air around the ant after a stimulation are also indicated.

The total intensity of the alarm response from the ants stimulated with an increasing amount of formic acid is shown in Fig. 2. The amounts used were 1, 2, 4, 8, and 16 cm³ saturated gas of formic acid. Four replicate experiments have been done with each amount. One cm³ of formic acid is obviously very close to the threshold value for formic acid under the experimental conditions. This means that the threshold value for the intense alarm behaviour is about $7 \cdot 10^{15}$ molecules cm⁻³ air. In calculating the figure the molecules of the stimulant are considered to be uniformly distributed in the test box. The threshold figure refers to the intense behavioural step No. 4. The threshold values for the first three alarm steps are lower.

As is also shown in Fig. 2 the total intensity of the alarm behaviour increases with the intensity of the stimulus. The intensity of the alarm behaviour is, however, not simply correlated with the number of molecules. The form of the dose-response curve is the same as that generally found in the electrophysiological response of the chemoreceptors on the antennae of insects when they are stimulated with various substances (DUMPERT 1972 b, KAFKA 1970, PRIESNER 1973).

Formic acid is the main component of the secretion from the poison gland in formicine ants (STUMPER 1952). In F. rufa this acid and water constitutes more than 99 % of the secretion and the concentration of the acid has been estimated to 61.1 - 65.1 % (OSMAN and BRANDER 1961). Formic acid is used by formicine ants as a defence substance. It simultaneously acts as an alarm pheromone in some species of Formica (MASCHWITZ 1964) and Camponotus (BERGSTRÖM and LÖFQVIST 1972 a). Formic acid is, on the other hand, not an alarm pheromone for ants of the genus Lasius (MASCHWITZ 1964, REGNIER and WILSON 1969, DUMPERT 1972 b) nor for a related species, Acanthomyops claviger (REGNIER and WILSON 1968). So far the behavioural threshold value for formic acid as an alarm pheromone has been unknown:

Saturated hydrocarbons as alarm pheromones

Substances present in amounts > 0.1 % of the secretion of Dufour's gland from F. rufa and their relative proportions are shown in Table 1. The dominating role of the saturated hydrocarbons is obvious, undecane and tridecane together forming almost 70 % of the secretion.

The effect of saturated hydrocarbons from tridecane and down to octane as

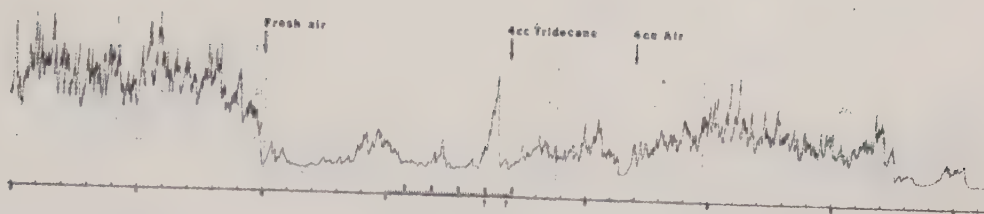
TABLE 1 - RELATIVE PROPORTION OF SUBSTANCES PRESENT IN $\geq 0,1\%$ IN THE SECRETION OF DUFOUR'S GLAND IN F. rufa.

Substance	Substance No. according to Table 2	<i>F. rufa</i> amount in %
Nonane	1	0,6
Decane	3	3,6
Undecene and undecane	4+5	51,2
5-Methylundecane	6	0,5
3-Methylundecane	7	1,6
Dodecene and dodecane	9+10	1,0
Tridecene	12	3,2
Tridecane	13	19,9
Pentadecene	20	0,2
Pentadecane	22	5,6
Tetradecenol and heptadecadiene	27+28	} 2,0
Heptadecene	29	
Heptadecane	30	6,2
Octadecane	34	0,1
Nonadecene	37	0,9
Nonadecane	38	0,3
Heneicosene	43	0,2
All-trans-geranylgeraniol	45	0,2
Octadecyl acetate and docosane	46+47	1,5
All-trans-geranylgeranyl acetate and tricosene	48+49	1,3

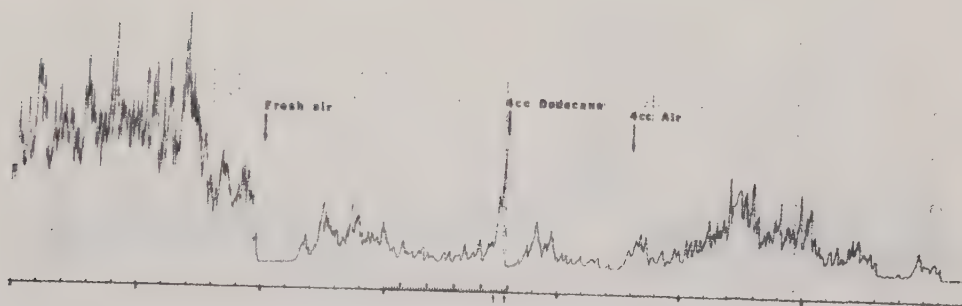
* Mass spectral data show that undecane constitutes $< 1\%$ of the total secretion.

The table is extracted from BERGSTRÖM and LÖFQVIST 1973.

a



b



c

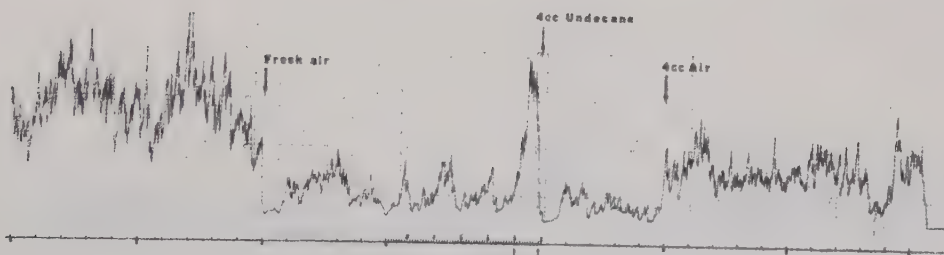
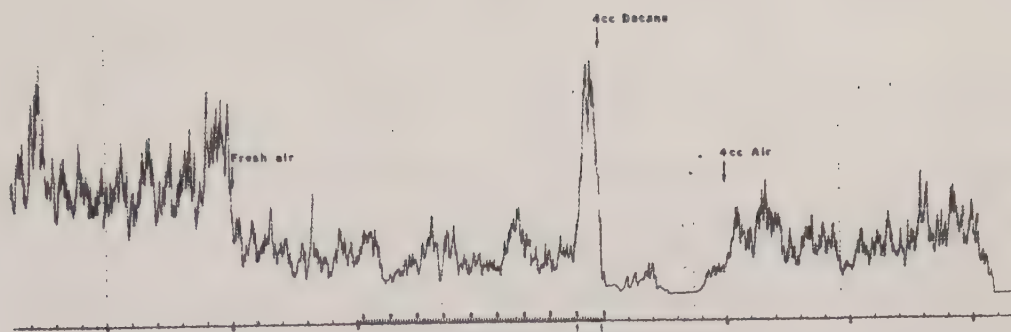
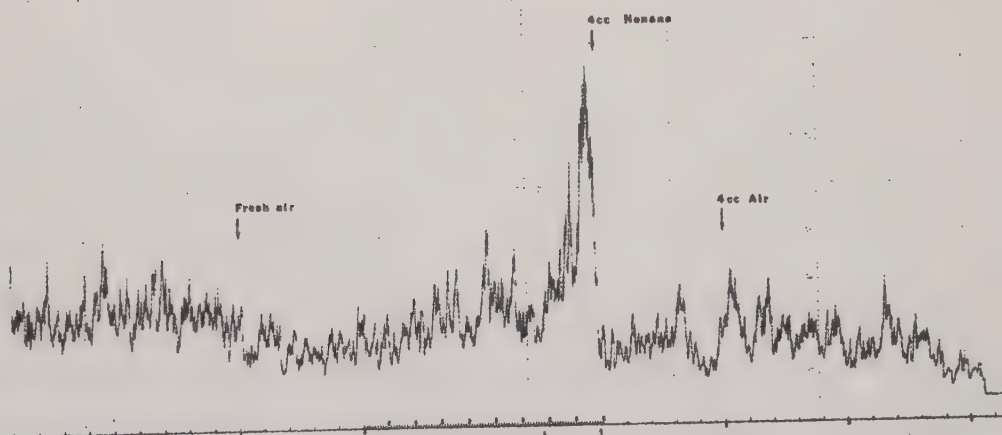


FIG. 3 a-f. Pen recorder diagrams of the locomotor activity of 20 worker ants of *F. rufa*. The ants have in each experiment been stimulated with a hydrocarbon from a homologous series of six aliphatic saturated hydrocarbons. For further explanations see Fig. 1.

d



e



f

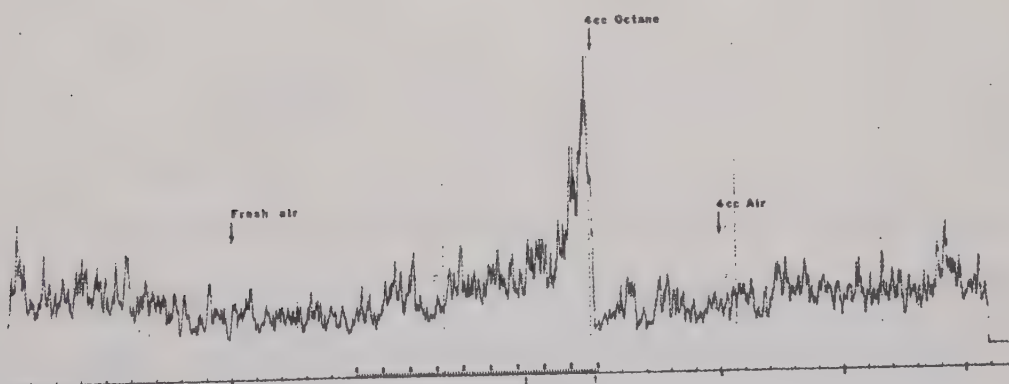


FIG. 3. (Contin.)

alarm pheromones for F. rufa is shown in Fig. 3 a-f. Four cm^3 saturated gas were used as a stimulus in each experiment. At least in this amount both tri-decane and dodecane are very weak alarm pheromones. Undecane, decane and nonane are in increasing order stronger alarm pheromones, whereas octane is a somewhat weaker alarm pheromone than nonane.

Four cm^3 saturated gas of nonane and octane is a rather high stimulus (Fig. 3). The ants do not therefore end the fast-running phase of their alarm behaviour at the same time. Some ants show a rather high locomotor activity up to several minutes after the initial fast-running phase. The locomotor activity of the whole group of ants decreases therefore gradually over a period of up to 10 min or even more after the stimulation.

The total intensity of the fast-running phase of the alarm behaviour in workers of F. rufa gradually increases when the ants are stimulated with 4 cm^3 of the hydrocarbons from dodecane down to nonane (Fig. 4 a). This increase is due to an increase in the maximum intensity as well as in the duration (Figs. 4 b, c)

The number of molecules (Fig. 4) is of course very different in 4 cm^3 saturated gas of the hydrocarbons examined. The connection between the number of molecules and the response intensity of different substances as alarm pheromones seems to be rather complicated. A comparison between the various hydrocarbons as alarm pheromones presupposes that the dose-response relationship has been worked out for each substance. The absolute values for different amounts of the test substance and the threshold value are figures necessary for such an evaluation. This will be dealt with in a following paper.

The dose-response curve for one hydrocarbon, decane, as an alarm pheromone for F. rufa is shown in Fig. 5. 0.25 cm^3 decane releases such a clear alarm behaviour of the ants, that the threshold value for decane must be lower. This amount corresponds to $6 \cdot 10^{13}$ molecules cm^{-3} air, if the molecules are uniformly distributed in the test box. The threshold value for decane as an alarm pheromone for F. rufa is thus more than a hundred times lower than the corresponding value for formic acid. On the other hand there are no saturation effects of a great amount of decane such as the 4 cm^3 that was used in a second series (Fig. 5).

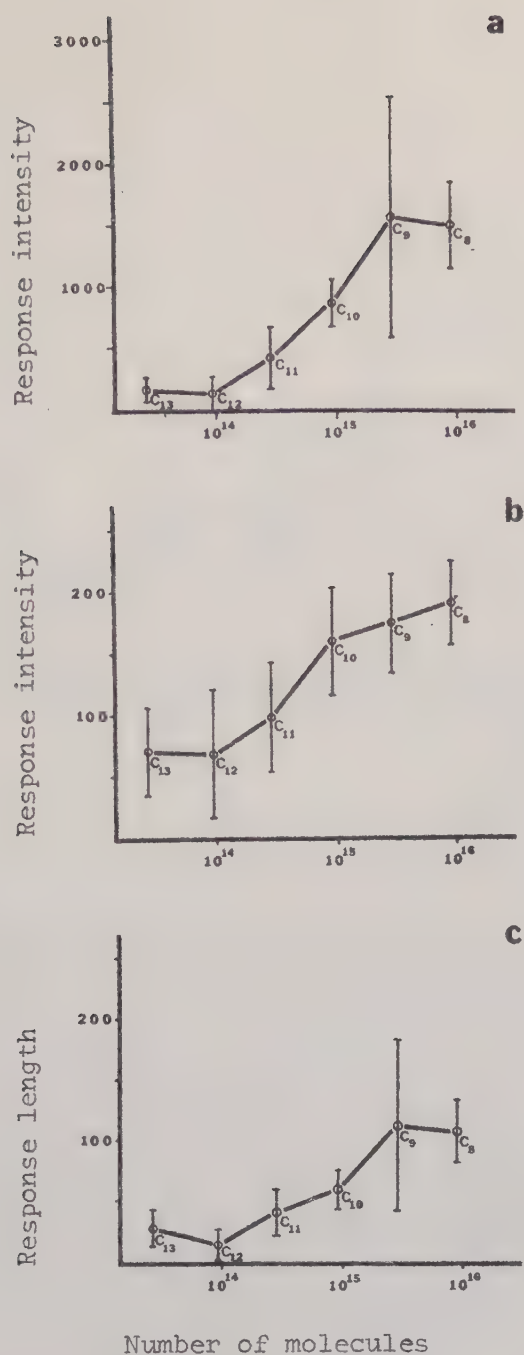


FIG. 4 a-c. Response curves of a/ the total intensity in pulses min^{-1} , b/ the maximum intensity in pulses 6 sec^{-1} , and c/ the duration in seconds of the fast-running phase of the alarm behaviour of 20 worker ants of *F. rufa* in a series of experiments with 4 cm^3 saturated gas of one of the saturated aliphatic hydrocarbons from C_{13} and down to C_8 as a stimulus. The standard deviation is shown for each value, which in this series is a mean value for eight experiments, all performed on the same day.

The estimated number of molecules cm^{-3} air around the ants after a stimulation is indicated on the abscissa.

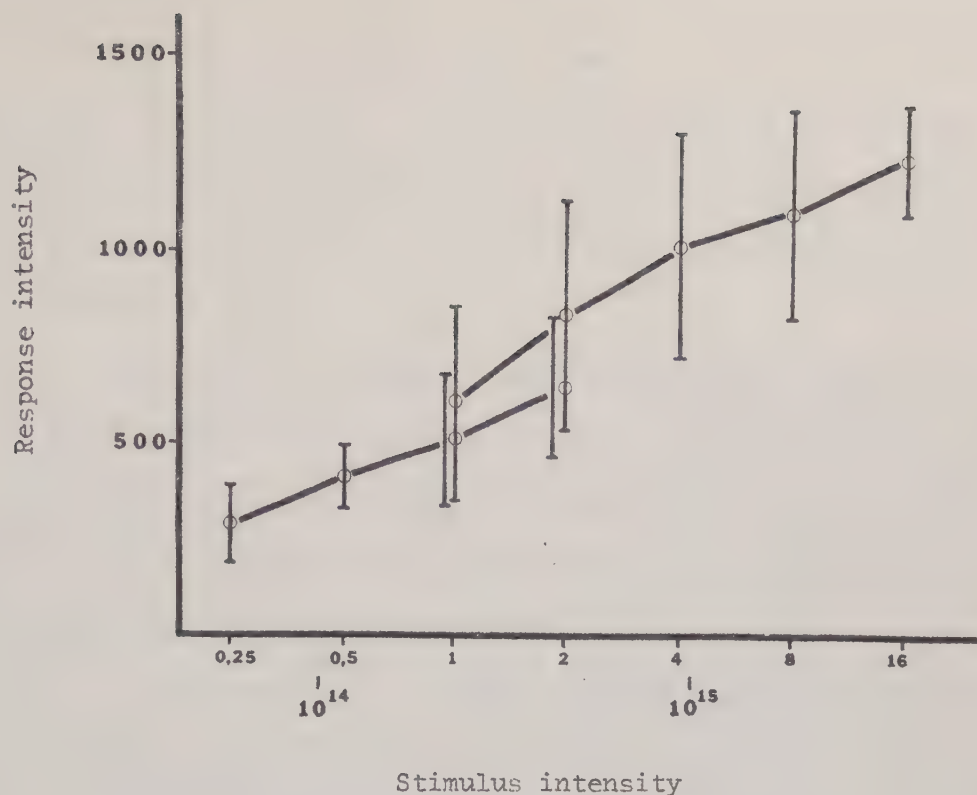


FIG. 5. Response curve shown as the total intensity of the fast-running phase of the alarm behaviour of 20 worker ants of F. rufa stimulated with n-decane as saturated gas in two series of experiments. In the first series - upper curve - the stimulus amount has been varied from 1 to 16 cm³, in the second series - lower curve - from 0.25 to 2 cm³. In the latter series decane has been diluted with spectroscopically pure paraffin oil in a proportion of 1:10. The standard deviation is shown for each value, which in the two series is a mean value for four experiments.

The designations of the figure are identical with those in Fig. 2.

Reaction groups

The specificity of insect receptors or receptor cells for chemical substances has been studied electrophysiologically for some insects (cf. KAISLING 1971, PRIESNER 1973, PAYNE 1974). A certain kind of receptor or receptor cell has been found to have a spectrum ^{of} substances to which the cell responds. Such a spectrum has been called a "reaction group" (KAFKA 1970). Different kinds of cells have different reaction groups.

It is well known that a chemoreceptor will be adapted for some time after it has been stimulated. This means that if it is stimulated with the same substance once again while it is to some degree adapted it will respond much less strongly than on the first occasion. In the method used in this investigation, unlike that involving electrophysiological measurements, all receptors or receptor cells are exposed to the stimulant, and it is not possible immediately to distinguish between different types of receptors. One kind of receptor or receptor cell can respond to one substance tested, while another kind of receptor cell responds to another substance. It would be valuable to know whether the substances tested belong to different reaction groups or not. This information can be attained by cross-testing two substances as follows.

In a first series of experiments (Tab. 2) the ants were stimulated with 4 cm³ saturated gas of formic acid. Twenty minutes later the ants were again stimulated with the same amount of the acid. The alarm behaviour from the second stimulation is only 21 % of the first one, which is explicable as an adaptation phenomenon. In experiments with 4 cm³ saturated gas of undecane the second stimulation resulted in a reduction in the intensity of the response down to 15 %. These experiments were followed by two in which the first stimulus was 4 cm³ saturated gas of formic acid and the second the same amount of undecane. In this case (Tab. 2), the reaction upon the second stimulus is not at all influenced by the first one. This means that formic acid and undecane belong to different reaction groups, probably perceived by different receptor cells.

The reaction groups for alarm substances have been studied with electrophysiology on the chemoreceptors in Lasius fuliginosus by DUMPERT (1972 b). He also found formic acid and saturated hydrocarbons to belong to different reaction

TABLE 2 - ADAPTATION EFFECTS OF FORMIC ACID AND UNDECANE AS RELEASERS OF THE FAST RUNNING PHASE OF THE ALARM BEHAVIOUR IN F. rufa.

	Stimulus 1 Formic acid	Stimulus 2 Formic acid	Number of exp- eriments	Stimulus 2 in % of stimulus 1	Stimulus 1 Undecane	Stimulus 2 Undecane	Number of exp- eriments	Stimulus 2 in % of stimulus 1	Stimulus 1 Formic acid	Stimulus 2 Undecane	Number of exp- eriments	Stimulus 2 in % of stimulus 1
Total intensity as counted pulses	767.7 ± 223.08	166.0 ± 59.57	3	21.6 ± 26.70	662.7 ± 101.76	98.0 ± 111.29	3	14.8 ± 109.37	371.5 ± 65.76	440.0 ± 138.59	2	118.4 ± 210.75
Maximum intensity as counted pulses	210.7 ± 90.74	64.3 ± 32.31	3	30.5 ± 35.61	164.0 ± 8.54	44.0 ± 18.35	3	26.8 ± 214.87	124.5 ± 68.58	173.5 ± 44.54	2	139.4 ± 64.95
Duration in seconds	52.0 ± 9.16	20.0 ± 3.46	3	38.5 ± 37.77	48.0 ± 0	16.0 ± 15.09	3	33.3	36.0 ± 16.97	33.0 ± 12.72	2	91.7 ± 74.96

groups, although this ant responds very weakly to formic acid.

Another series of experiments was carried out with 4 cm³ saturated gas of undecane as the first stimulus, followed 20 min later by a second stimulus of 4 cm³ saturated gas of one of the hydrocarbons from tridecane and down to octane. Three replicate experiments have been done with each hydrocarbon. The mean values of the total intensity, maximum intensity and duration for the alarm behaviour from the second stimulus of these experiments are shown in Figs. 6 a-c. For comparison a series of experiments is shown with eight replicates of the same hydrocarbons in the same amounts. This series was run on the second instrument at the same time. The ants in the two series therefore have the same physiological background, and the results can be compared. As regards the total intensity of the alarm behaviour in the two series (Fig. 6 a) the second stimulation is weaker than the first one. The lower response intensity is more pronounced in the maximum intensity values of the alarm behaviour (Fig. 6 b). Obviously undecane as a first stimulus blocks the perception of all the saturated hydrocarbons tested. Probably all the hydrocarbons belong to the same reaction group. DUMPERT (1972 b) also found that saturated hydrocarbons which he used as stimuli for the receptors belonged to the same reaction group.

Combined effect of two substances from different reaction groups as alarm pheromonones

In Figs. 7 a-c curve No. I represents hydrocarbons tested as separate substances, while curve No. II shows the combined effect of the hydrocarbon and formic acid. There is a high consistency in the total intensity of the alarm behaviour between the two series (Fig. 7). It is true that the combined effect of a hydrocarbon and formic acid is always higher than the effect of the hydrocarbon alone, but the difference between these two values is rather constant. There is also a rather constant difference in the duration of the fast-running phase of the alarm behaviour between the two series (Fig. 7 c). The maximum intensity on the other hand shows another pattern (Fig. 7 b) : it increases from tridecane to dodecane in combination with formic acid. More importantly the maximal intensity is rather constant and very high for the rest of the hydrocarbons in their combination with formic acid. The two series show that the hydrocarbons when com-

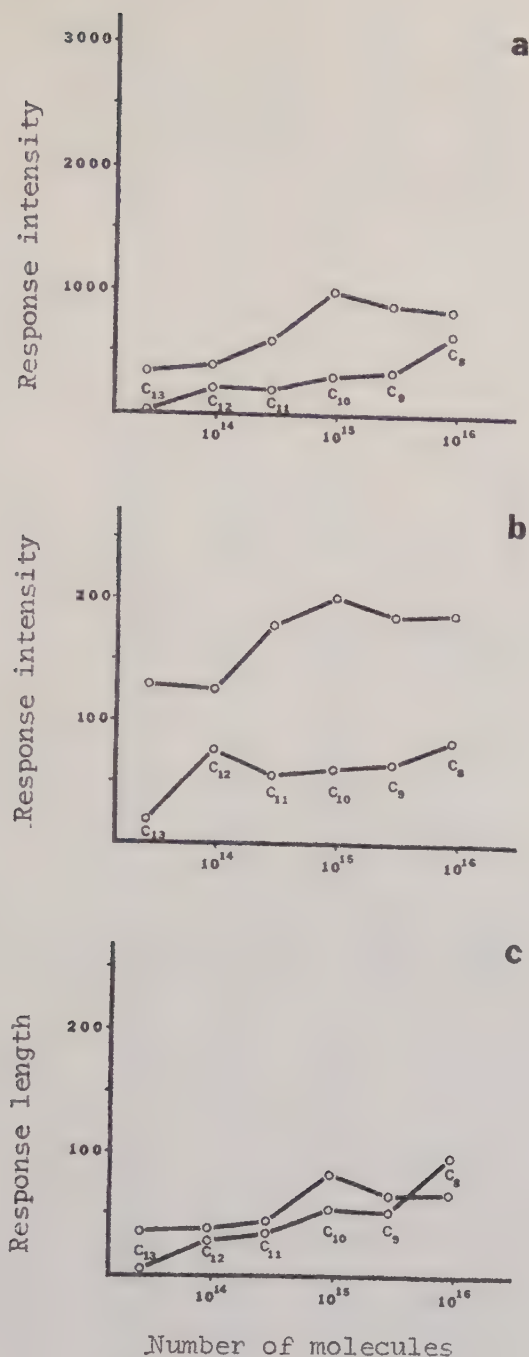


FIG. 6 a-c. Response curves of a/ the total intensity in pulses min^{-1} , b/ the maximum intensity in pulses 6 sec^{-1} , and c/ the duration in seconds of the fast-running phase of the alarm behaviour of 20 worker ants of *F. rufa* in two series of experiments with 4 cm^3 saturated gas of the saturated aliphatic hydrocarbons from C_{13} and down to C_8 as a stimulus. The substances used as stimuli, are shown below the lower curves. These show the adaptation effects when the ants are stimulated with 4 cm^3 saturated gas of undecane 20 min before the second stimulation with one of the various hydrocarbons. The values are mean values from three consecutive experiments. The upper curve shows the results when the ants are stimulated with the hydrocarbons as the first stimulus. The values are mean values for eight experiments, all performed on the same day.

The designations are identical with those in Fig. 4.

TABLE 3 - THE COMBINED EFFECTS OF FORMIC ACID AND TRIDECANE AS RELEASERS OF THE FAST RUNNING PHASE OF THE ALARY BEHAVIOUR IN P. rufa.

TOTAL INTENSITY AS COUNTED PULSES DURING THE DURATION.

	Series II in Fig. 7.		Series IV in Fig. 7.			Series III in Fig. 7.		Series I in Fig. 7.		Series III + I	
	$\bar{x}$	s	$\bar{x}$	s		$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
3 cm ³ formic acid + 4 cm ³ tridecane	703.5	±311.93	677.1	±303.20	3 cm ³ formic acid	555.8	±136.64	4 cm ³ tri- decane	163.3	±104.16	719.6
3 cm ³ formic acid + 4 cm ³ dodecane	943.4	±144.41	975.0	±391.63	8 cm ³ formic acid	518.3	±219.37	4 cm ³ do- decane	134.1	±140.35	652.4
3 cm ³ formic acid + 4 cm ³ undecane	1087.4	±169.97	976.7	±239.94	8 cm ³ formic acid	468.6	±159.95	4 cm ³ un- decane	425.6	±255.53	394.2
3 cm ³ formic acid + 4 cm ³ undecane			1048.1	±219.67	8 cm ³ formic acid	579.9	±228.67	4 cm ³ un- decane			
3 cm ³ formic acid 4 cm ³ decane	1476.3	±253.41	1307.0	±425.27	8 cm ³ formic acid	622.8	±195.21	4 cm ³ decane	330.6	±208.63	1453.4
3 cm ³ formic acid + 4 cm ³ nonane	7052.0	±639.58	2187.9	±350.78	8 cm ³ formic acid	585.3	±314.43	4 cm ³ nonane	1562.3	±981.58	2147.6
3 cm ³ formic acid + 4 cm ³ octane	1653.9	±725.58	1642.6	±366.71	8 cm ³ formic acid	550.6	±113.70	4 cm ³ octane	1495.1	±354.27	2045.7

TABLE 3 (Contin.)

MAXIMUM INTENSITY AS COUNTED PULSES DURING SIX SECONDS.

	Series II in Fig. 7.		Series IV in Fig. 7.		Series III in Fig. 7.		Series I in Fig. 7.		Series III + I
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	
8 cm ³ formic acid + 4 cm ³ tridecane	113.2	± 35.00	107.5	± 37.58	114.5	± 27.13	71.4	± 36.13	135.9
8 cm ³ formic acid + 4 cm ³ dodecane	157.2	± 19.31	158.6	± 59.42	124.2	± 52.39	69.0	± 52.63	193.2
8 cm ³ formic acid + 4 cm ³ undecane	150.5	± 29.00	141.3	± 40.30	122.8	± 42.20	99.0	± 45.19	221.8
8 cm ³ formic acid + 4 cm ³ undecane	175.6	± 44.56	137.8	± 59.23	133.5	± 30.05	160.8	± 44.04	294.3
8 cm ³ formic acid + 4 cm ³ nonane	172.8	± 36.59	152.9	± 44.45	126.9	± 39.52	175.3	± 40.74	302.2
8 cm ³ formic acid + 4 cm ³ octane	168.1	± 59.59	164.4	± 38.82	101.2	± 23.05	191.8	± 34.67	293.0

TABLE 3 (Contin.)

DURATION IN SECONDS.

	Series II in Fig. 7.		Series IV in Fig. 7.		Series III in Fig. 7.		Series I in Fig. 7.		Series III + I	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	
8 cm ³ formic acid + 4 cm ³ tridecane	75.0	± 21.03	74.2	± 25.84	56.3	± 11.08	4 cm ³ tri- decane	27.8	± 15.35	84.1
3 cm ³ formic acid + 4 cm ³ dodecane	80.2	± 12.80	78.8	± 14.87	50.3	± 8.44	4 cm ³ do- decane	14.3	± 12.80	64.6
3 cm ³ formic acid + 4 cm ³ undecane	96.0	± 34.54	79.7	± 8.97	37.5	± 7.69	4 cm ³ un- decane	41.3	± 19.09	78.8
8 cm ³ formic acid 4 cm ³ undecane			96.8	± 19.62	51.8	± 7.12	4 cm ³ un- decane			
8 cm ³ formic acid + 4 cm ³ decane	111.0	± 21.98	115.0	± 29.73	53.3	± 15.52	4 cm ³ de- cane	60.0	± 16.24	113.3
8 cm ³ formic acid + 4 cm ³ nonane	160.5	± 46.88	168.8	± 55.40	47.3	± 22.32	4 cm ³ non- ane	113.1	± 70.69	160.4
3 cm ³ formic acid + 4 cm ³ octane	146.2	± 50.29	130.5	± 31.38	57.0	± 14.69	4 cm ³ oc- tane	108.0	± 26.05	165.0

bined with formic acid affect the duration of the response and thereby also the total intensity of the alarm behaviour. It also seems that the total intensity and the duration for the alarm behaviour is purely additive for a hydrocarbon and formic acid when they are combined.

This combined effect of a hydrocarbon and formic acid on the total intensity and duration of the alarm behaviour has been further investigated in two series of experiments shown in Fig. 7 d-f. In the figure curve No. III represents formic acid tested as a separate substance, and curve No. IV shows the combined effect of the various hydrocarbons with formic acid. There is a great similarity between the results shown in curve No. IV and the corresponding ones from the first experimental series shown in curve No. II. This indicates that the animal material is very uniform in all these four series and that the results can be compared quantitatively.

It is seen from series No. III and IV that the maximal intensity of the alarm behaviour (Fig. 7 e) does not increase if the ants are stimulated with hydrocarbons combined with formic acid compared to stimulation with formic acid alone.

The results from the four experimental series have been summarized in Tab. 3. When the figures of the total intensity and duration for the alarm behaviour are added from the pure hydrocarbon series (No. I) and from the pure formic acid series (No. III) the figures thus obtained are very similar to the corresponding ones for the combination of the substances (Nos II and III). This means that the total intensity and duration of the fast-running phase is a simple addition of the values for saturated hydrocarbons and formic acid.

Combined effect of two substances from the same reaction group as alarm pheromones

The alarm-defence secretion from Dufour's gland in F. rufa has been found to be composed of saturated hydrocarbons. These saturated hydrocarbons seem to belong to the same reaction group. In the light of the additive effect of formic acid and a hydrocarbon it would be interesting to look at the effect of two combined saturated hydrocarbons. In such an experiment (Tab. 4) the alarm stimulation effects have been compared between 17 cm³ undecane combined with 8 cm³ formic acid with those from 4 cm³ undecane plus 4 cm³ decane combined with 8 cm³

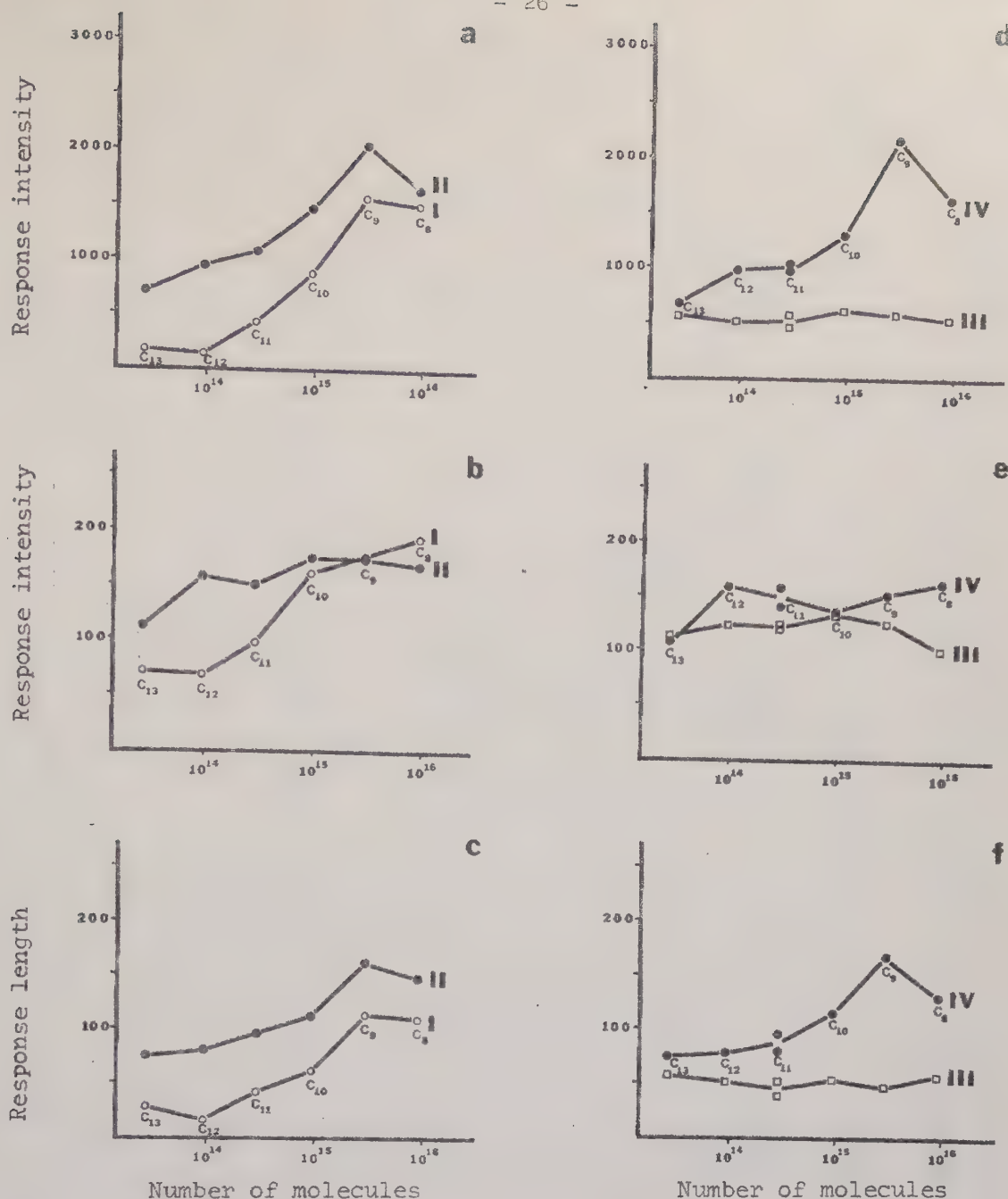


FIG. 7 a-f. Response curves of a/ and d/ the total intensity in pulses min^{-1} , b/ and e/ the maximum intensity in pulses 6 sec^{-1} , c/ and f/ the duration in seconds of the fast-running phase of the alarm behaviour of 20 worker ants of *F. rufa* in four series of experiments. The stimulus in series I has been 4 cm^3 saturated gas of the hydrocarbons indicated in the figure. The same stimuli have been used in series II but 8 cm^3 saturated gas of formic acid have at the same time been injected from a second syringe. The importance of the different hydrocarbons for the duration and the total intensity of the behavioural step is clearly shown. The second series has been run at the same time as the experiments in the first series but on a second instrument.

Series IV is identical with the second, but was carried out some days later. The great similarity of the results from the two series is evident. Series III is a control series carried out on a second instrument at the same time as the fourth series and in which the ants were stimulated with 8 cm^3 saturated gas of formic acid.

Each value shown in the four series is a mean value of eight experiments, all performed on the same day. In series III and IV 2×8 experiments have been done with formic acid and undecane respectively, eight of them five days later than the first eight. The small differences in the results from the two days indicate that the animal material has been very uniform during the two series (III and IV).

The standard deviation of the experiments in curve I is shown in Fig. 4. The designations in the figure are identical with those in Fig. 4.

TABLE 4 - THE EFFECT OF TWO HYDROCARBONS COMPARED TO A COMBINATION OF ONE HYDROCARBON AND FORMIC ACID AS RELEASERS OF THE FAST-RUNNING PHASE OF THE ALARM BEHAVIOUR IN F. rufa.

Date of experiments	Stimulus: 8 cm ³ Formic acid + 17 cm ³ undecane				Stimulus: 8 cm ³ Formic acid + 4 cm ³ undecane + 4 cm ³ decane			
	Number of experiments	Total intensity $\bar{x}$ s	Maximum intensity $\bar{x}$ s	Duration $\bar{x}$ s	Number of experiments	Total intensity $\bar{x}$ s	Maximum intensity $\bar{x}$ s	Duration $\bar{x}$ s
13 Oct. 1975	4	936.0 ±394.47	135.8 ±29.65	73.5 ±29.54	4	1846.5 ± 802.87	137.0 ±18.31	139.5 ±53.07
14 Oct. 1975	3	1642.3 ±824.81	146.5 ±28.16	114.0 ±43.26	4	2162.0 ± 989.82	203.0 ±38.45	124.5 ±59.67
15 Oct. 1975	3	1648.3 ±407.13	150.3 ± 5.50	112.0 ±39.94	4	3359.0 ± 296.18	172.0 ±10.61	193.5 ±22.64
16 Oct. 1975	4	1429.5 ±811.62	159.5 ±22.86	96.0 ±48.24	3	3197.3 ±1742.04	191.3 ±53.78	186.0 ±38.38
17 Oct. 1975	4	1862.5 ±216.11	156.0 ±14.98	118.5 ±19.82	4	2131.2 ±1000.11	182.2 ±49.22	147.0 ±56.81
	18	$\bar{x}_{18}$	$\bar{x}_{18}$	$\bar{x}_{18}$	19	$\bar{x}_{19}$	$\bar{x}_{19}$	$\bar{x}_{19}$
		1438.0 ±604.75	148.8 ±22.35	101.7 ±36.72		2504.6 ±1088.46	176.4 ±39.97	156.6 ±57.58

formic acid. If the number of molecules contained in 17 cm^3 saturated gas of undecane is assumed to be uniformly distributed in the test box it may be estimated to $120.7 \cdot 10^{13}$ molecules cm^{-3} . The corresponding figure for 4 cm^3 undecane combined with 4 cm^3 decane is $121.6 \cdot 10^{13}$. The two stimuli thus contain about the same number of molecules. The hydrocarbon(s) have in all experiments been combined with formic acid because this is the natural situation for the ants. The different substances have always been injected as saturated gases at the same time but from different syringes.

The results (Tab. 4) show that the total intensity of the alarm behaviour is about 70 % higher in a combination of decane and undecane compared to a stimulus of undecane only, although the two stimuli contained the same number of molecules. This effect is obviously due to an increased duration, while the maximum intensity differs only slightly. It seems that one of the advantages of the many saturated hydrocarbons in the complex alarm-defence secretion in F. rufa is to release an intense alarm reaction in the ants for a longer time than would be possible with a single substance.

DISCUSSION

The pheromone secretion from Dufour's gland has been chemically analysed from about 20 species of ants. The secretion has been found to contain several substances, sometimes 40 or more (BERGSTRÖM and LÖFQVIST 1968, 1970, 1972 a, 1972 b, 1973, BROPHY et al. 1973, CAVILL and WILLIAMS 1967, REGNIER et al. 1973, REGNIER and WILSON 1968, 1969). The majority of the substances in the secretion from a species form one or several homologous chemical series. In formicine ants the quantitatively dominating series consists of saturated aliphatic hydrocarbons. Sometimes there is also a series of unsaturated aliphatic hydrocarbons and/or methyl branched hydrocarbons. There is very often one series of ketones, acetates, or alcohols. In addition there are very often some single isoprenoids.

The substances from Dufour's gland in F. rufa are most probably emitted together with formic acid as an alarm-defence signal. From the secretion of the gland in this species 41 substances have been identified, 11 of which are present

in amounts exceeding 1 % of the secretion (BERGSTRÖM and LÖFQVIST 1973). The secretion can theoretically function as a mode of communication based on quality and quantity for each of all the substances. At the same time the information is dynamic, because all the substances are volatile, so that their relative proportions will be changed by degrees as the more volatile substances evaporate below the concentration that can be perceived by the animals.

How much of the potential information of the secretion is exploited by the animal ? The biological situation is that an ant attacked by an enemy must be able to alarm and warn other ants from its nest about the danger threatening the nest, the superorganism. At a superficial consideration this message could be mediated by a single substance. The alarm defence behaviour in ants is, however, not a single behaviour but a behavioural chain consisting of a number of steps. "Alertation", attraction, fast-running, attack, are some of the steps, which may be involved. Many of the steps are composed by some behavioural patterns. Alertation f.i. can mean that the animal ceases to walk, raises and moves the antennae, turns towards the odour source, etc. Various behavioural patterns in the alarm-defence behaviour of the ants have been described (WALLIS 1962 a, 1962 b, WILSON 1962 1971, REGNIER and WILSON 1968, and ROBERTSON 1971). A behavioural chain such as the alarm-defence behaviour in ants cannot run to completion automatically only through releasing the first simple step. Evolution has avoided such snowball effects by continuously necessitating new stimuli for releasing the next steps in the chain. This is one reason why there is such a rich information in the chemical signal.

Three qualifications of the chemoreceptors are important if the potential information in the chemical signal should be capable of being exploited by the animals:

1. The chemoreceptors should be sensitive for the various substances.
2. The chemoreceptors must have a threshold value for the various substances adjusted to the very low amount in which these are usually present in the natural secretion.
3. The chemoreceptors must be able to perceive the concentration of a substance and its variation in the air.

The amount of information in the system is considerably increased if the animal can remember the variations in concentration of the different substances in time.

The first of these three points is comparatively well known in social hymenoptera. These have on their antennae a great number of receptors of a few different morphological types (DOSTAL 1958, KAISSLING 1971, PRIESNER 1973). The morphology of the receptors on the antennae in ants has recently been studied in Formica pratensis (KURSCHNER 1969) and in several ant species, especially Lasius fuliginosus by DUMPERT (1972 a) who in this species distinguishes seven kinds of receptors. Only one of these, however, was found to respond to odour stimuli. This receptor type reacted on a wide spectrum of odour substances, which could be arranged in ten reaction groups (DUMPERT 1972 b). Also another social insect, the honey bee, is capable of perceiving a large number of odours (VARESCHI 1971, KAISSLING 1971, MARTIN 1974, BITTEL and MARTIN 1974 and references therein). Social hymenoptera are in this respect very different from e.g. moths. The male ^{worm} silk moth, Bombyx mori, has about 16 000 chemoreceptors on each antenna. Each of the chemoreceptors has at the most two neurones, making a maximum number of 32 000 sensitive neurones. Eighty percent or 25 000 of these are receptors specialized for the female substance (KAISSLING and PRIESNER 1970). This species like most other moths can detect only a very few odour substances. The difference between moth and social hymenoptera reflects the difference between the great number of life situations in which the social hymenopteran are depending on odour stimuli compared to moths.

This investigation shows that all hydrocarbons tested are perceived. Octane, nonane, decane, and undecane release the intense fast-running phase of the alarm behaviour. Corresponding results have been found by REGNIER and WILSON (1968) for another formicine, Acanthomyops claviger. The same behavioural step in F. rufa is released also by formic acid as shown by MASCHWITZ (1964). This behavioural step is released by several substances.

The threshold value was mentioned above as the second qualification of the chemoreceptors necessary for the animals if they should be able to use the rich information in a complex signal. The threshold value of the chemoreceptors for a specific substance must always be adapted to the nature of the specific behaviour.

step, which that substance releases as well as to the amount of the substance given off. The threshold values for e.g. the fast-running phase of the alarm behaviour must not be too low. Low threshold values would mean that the signal given off by a single ant would be able to stimulate other individuals for a rather long time, which would entail an unnecessarily great waste of energy (WILSON and BOSSERT 1963). Very few threshold values have been determined for steps in the alarm-defence behavioural chain of formicine ants. REGNIER and WILSON (1968) reported the behavioral threshold values for the fast-running phase of the ant Acanthomyops claviger for a series of saturated aliphatic hydrocarbons. DUMPERT (1972 b) determined the threshold value for the receptor in Lasius fuliginosus for a few substances. The threshold values for all the various substances tested in this investigation have not been determined but values are given for one hydrocarbon, decane, and for formic acid. The behavioural threshold for releasing the fast-running phase for different substances has in different investigations been found to be in the same range, $10^{12} - 10^{16}$ molecules cm^{-3} air. For pheromones this is a high threshold value, but considering the nature of the behavioural step this was predicted to be the case by WILSON and BOSSERT (1963).

The ability of the ants to perceive the concentration and concentration variations of the various substances was pointed out above as the third qualification of the chemoreceptors necessary if they should be able to use the rich information in the complex volatile secretion. They can probably do this. It is presumed that the stronger the concentration, the more acceptors (KAFKA 1970) are stimulated. WILSON (1958) found that two behavioural steps, "alarm behaviour" and "digging behaviour" are released by the same substance in the ant Pogonomyrmex badius. The second behaviour pattern is released by a higher concentration of the stimulus.

Returning to Dufour's gland in F. rufa its secretion is dominated by one homologous series of chemical substances. The substances in a series of homologues may be able to release the same behavioural step. The most volatile components release the step immediately, the less volatile substances are active when the concentrations of the former have fallen below the threshold values by evaporation. Such a system adjusts the rapidity of the release as well as the duration of the reaction. Homologues are also able to co-operate in order to release a

high intensity of a behavioural step. For a species responding to a single substance only, the threshold value for the substance must be very low when the same rapid release is to be obtained. Besides, a single substance cannot regulate the duration of the reaction, a short-coming which has many disadvantages as was pointed out above. The value of a combination of homologous substances for regulating the duration of a behavioural step was shown above (p. 28): the same number of molecules as a stimulus was compared for undecane and for a combination of undecane and decane. The combination of the two substances released the behaviour with a slightly higher maximum intensity than for undecane as a single stimulus but gave a longer duration.

The isolated substances in a homologous series may also release different behavioural steps. In an alarm-defence behavioural chain the most volatile substances release "alerting". Substances of a medium volatility may release the "fast-running phase", i.e. the most intensive part of the behavioural chain. Substances of lower volatility finally may release the same behavioural step in a much lower intensity and might be regarded as a kind of stop signals for that step. Such a pattern has been observed in F. rufa (LÖFQVIST unpubl.) and discussed earlier (BERGSTRÖM and LÖFQVIST 1970, 1972 a). When studying the electropotentials of the receptor cells in Lasius fuliginosus, DUMPERT (1972 b) found that tridecane was one of the very few substances that suppress the normal impulse frequency of the cells. This indicates that tridecane can be a stop signal for the alarm-defence behaviour in L. fuliginosus.

If the substances in a homologous series release different steps in a behavioural chain, it is easy to realize that less closely related substances are able to do the same. This has recently been shown for the African weaver ant Oecophylla longinoda (BRADSHAW et al. 1975). In this ant "hexenal releases alerting and alarm with little evidence of directional orientation" while "1-hexanole attracts ants towards it". The biting behaviour is controlled by two substances, 3- undecanone and 2-butyl-2-octenal. It is important to note that the function of the different substances is coordinated with their volatility : hexenal is the most volatile and 2-butyl-2-octenal is the least volatile. Without knowing the chemistry of many of the substances ROBERTSON (1971) worked with alarm-defence behavioural steps and showed that different steps are re-

leased by secretions from the different glands, but also that sometimes a step may be released by the combination co-operation of secretions from two glands.

The present investigation shows that the fast-running phase of the alarm-deference behaviour in workers of F. rufa can be released by formic acid as well as by many of the hydrocarbons from Dufour's gland. The formic acid and one or more hydrocarbons can act together and release a stronger behaviour which in this case means only slightly higher intensity but longer duration. When acting together the effect of formic acid and a hydrocarbon is purely additive. This may be explained in accordance with (KAFKA 1970) : the more acceptors that are activated the stronger the behaviour.

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TOXIC PROPERTIES OF THE CHEMICAL DEFENCE SYSTEM IN FORMICA RUFA L. AND
FORMICA SANGUINEA LATR., TWO COMPETITIVE SPECIES

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Abstract Formic acid and substances from Dufour's gland constitute the chemical defence system in F. rufa and F. sanguinea. The secretion from Dufour's gland consists in F. rufa mainly of hendecane and tridecane and in F. sanguinea mainly of hendecane, decyl acetate, and dodecyl acetate. The hydrocarbons at least are toxic for the ants also in small amounts. More importantly, however, the hydrocarbons and the acetates act as wetting agents for formic acid, which alone is rather harmless. There are only minor differences between the hydrocarbons and the acetates as wetting agents for formic acid. The substances kill the ants by penetrating their tracheal system, thus easily reaching the vital organs.

The hydrocarbons and the formic acid of F. rufa are more toxic for both species than the substances from F. sanguinea. F. sanguinea also seems to be more sensitive than F. rufa to the defence substances from both of the species. The reason may be its smaller size.

Differences in the composition of the secretion of Dufour's gland in the two species do not seem to explain the superiority of F. sanguinea in nest competition with F. rufa observed in the field. It may be explained by higher aggressiveness in F. sanguinea.

INTRODUCTION

Formica sanguinea Latr. is a slave-holding ant, which raids nests of mostly F. fusca L. and F. rufibarbis F. for slaves. It is a very aggressive ant which does not tolerate other Formica-species in the vicinity of its own nest. It is especially aggressive towards F. rufa L. According to MARIKOVSKY (1963) "the issue of the raid on the mound of F. rufa is invariably complete annihilation of all the inhabitants" and F. sanguinea often takes over nests of F. rufa. I have also had the opportunity of observing nest competition between the two species. In a study of ant colonization of a cleared part of a mixed forest, F. sanguinea was observed to take over a 3-4 year-old and vigorous nest of F. rufa and also some weaker nests. In some cases F. rufa took over weak nests of F. sanguinea (LÖFQVIST unpubl.).

The most important defence of formicine ants consists of formic acid and substances from Dufour's gland, which are sprayed on the enemy. Formic acid is produced by a gland and stored in a vesicle, which is emptied by muscular contractions of the gaster. More than 99 % of the secretions is made up by formic acid in a concentration of about 60 % (OSMAN and BRANDER 1961). Dufour's gland is a small gland opening in the duct from the poison vesicle in the tip of the gaster (cf. BERGSTRÖM and LÖFQVIST 1973). In formicine ants both glands are emptied at the same time in defence (REGNIER and WILSON 1968).

In formicine ants the secretion from Dufour's gland is built up of numerous substances, sometimes more than 40, most of which are present in trace amounts only. We have identified the major substances from the gland in workers of F. sanguinea (BERGSTRÖM and LÖFQVIST 1968) and most substances in workers of F. rufa (BERGSTRÖM and LÖFQVIST 1973). The major substances in F. sanguinea were found to be hendecane, decyl acetate, and dodecyl acetate, and in F. rufa hendecane and tridecane. The acetates give F. sanguinea a different defence secretion from that of F. rufa. The toxicity and the role in the defence of the two species of the various substances have been investigated. The importance of substances present in Dufour's gland for the nest competition between the two species is analysed.

MATERIALS

Worker ants of F. rufa were collected from two nests in southern Sweden, one in Skogsby, Öland and the other in Björnstorp, Skåne. To judge from their pilosity, the ants from both nests were typical F. rufa (LANGE 1958, BETREM 1960). The two nests were probably monogynous because they were inhabited by rather few ants most of which were of the same large size. Big nests of F. sanguinea are rare in Sweden, so material of this species was collected from ten nests in southern Sweden at Skogsby, Stora Rör, and Norra Möckleby, all on Öland, Haverdalsstrand in Halland, Lessebo and Linneryd in Småland, and Skärvalid in Skåne.

METHODS

Conditions for the ants

In order that the ants should not be exposed to their own defence substances, they were picked up very carefully individually on a stick from the surface of their nest and placed in a nest of plaster (FIG. 1). The relative humidity in these nests can be kept at more than 90 % and ants can live there for more than a month. For the present investigation new ants were collected every week and always in Saturdays or Sundays for the following week's experiments. The ants were fed sugar and water.

All experiments were done at a constant temperature of $23.0 \pm 0.2^{\circ}\text{C}$ and a relative humidity of $80 \pm 4\%$.

Performance of the experiments

When they attack F. rufa and F. sanguinea seize a hostile ant with their jaws and spray their defence substances on the enemy while holding it in a leg, an antennae etc. In doing so they rarely damage the sclerotized integument. In the experiments each ant was seized with forceps in the tibia of the mesothoracic legs and stretched out in such a way that it was unable to touch the forceps nor the table below with any part of its body (FIG. 2). Ants held in this way could move head, gaster, and kick with free legs attempting to get free. The thorax is, however, held so that test substances applied on the top of it

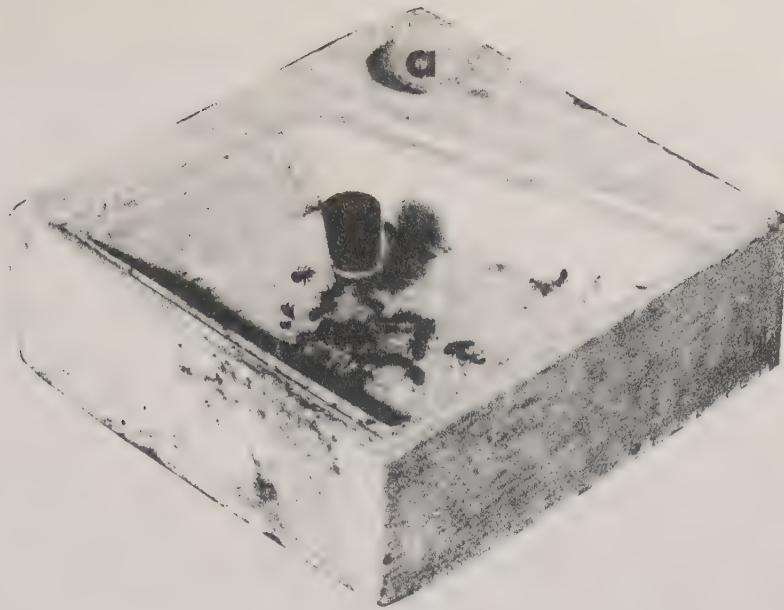


FIG. 1. An artificial ant nest of plaster. For each experiment 80 worker ants were collected and preserved in such a nest. Water was added through the hole (a) each day for keeping the nest air almost saturated.

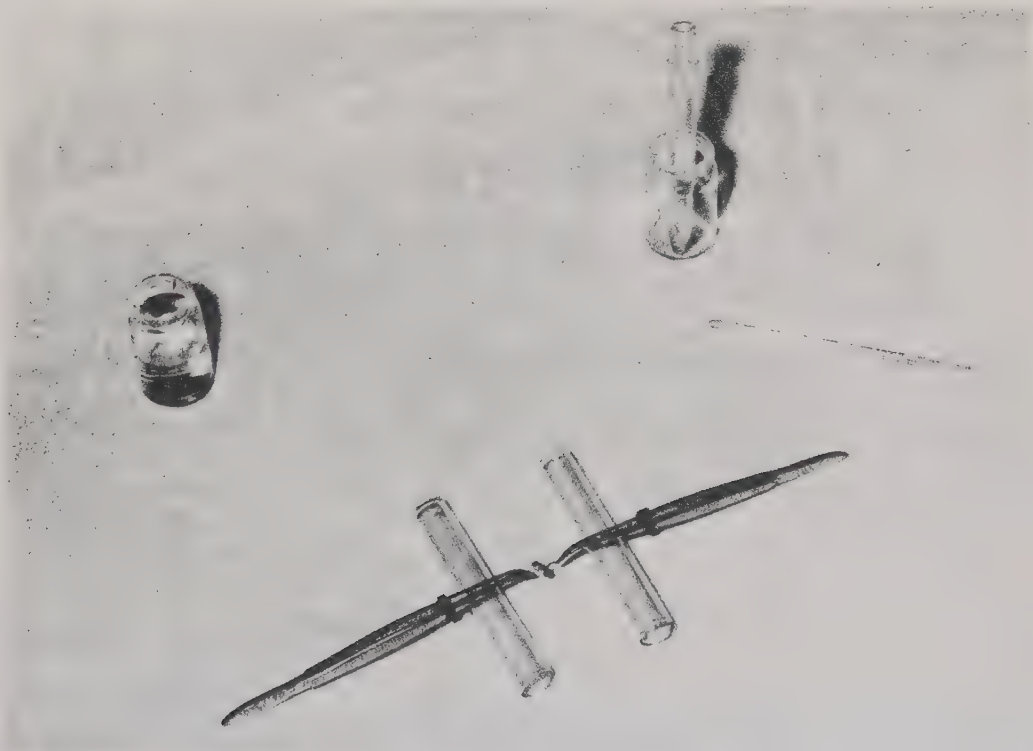


FIG. 2. A worker ant of F. rufa fixed with two forceps. The test substance was applied on the ant's thorax with a Carlsberg glass micropipette. To the right is a vial with test substances and to the left a bottle with diethyl ether for cleaning the forceps.

with a micropipette (Carlsberg glass pipette) do not spread beyond it. In order to avoid substances being transferred from one experiment to another the forceps were washed in diethyl ether before a new ant was captured. The time to death of the ant was measured in seconds with a stopwatch. The last movement of the ant was taken as indicating death.

The influence of the substances tested was registered for 10 minutes in F. sanguinea, which reacted fast, and for 15 minutes in F. rufa. The evaluation of the material has been done in such a way that the difference does not affect the results when comparing the two species.

The size of the ants is of crucial importance, for which reason only individuals of medium size were collected in the field. 80 ants were kept in each plaster nest. One such group of ants were used each day. In each of these groups the smallest and the largest individuals were rejected. The ants could not be weighed after the experiments because they were still covered with the test substances. The minimum distance between the eyes has instead been measured for each ant. The linear relation between the body weight and the minimal eye distance is illustrated in FIGS. 3 and 4.

In one experiment the tracheal openings on thorax were sealed with bee wax. After the ants had been anaesthetized with carbon dioxide bee wax was applied under a binocular microscope with a fine steel nib inserted in the tip of a heated soldering-iron. The heat of this was adjusted to the melting point of bee wax. It was in this way possible to seal the thoracic tracheal openings of the ants with a wax droplet covering only the opening. After sealing and anaesthesia most ants behaved normal. A few which did not were not used for the experiments.

Test substances

The same substances and substance combinations were tested on F. rufa and F. sanguinea. The following substances were used: n-hendecane, n-tridecane, n-hendecane and n-tridecane in a proportion of 9:1, n-decyl acetate, n-dodecyl acetate, n-hendecane + n-decyl acetate + n-dodecyl acetate in the proportions of 24:35:41, and formic acid diluted in water to a concentration of 60 %. The substances were tested in the following amounts: 5 μ l, 2 μ l,

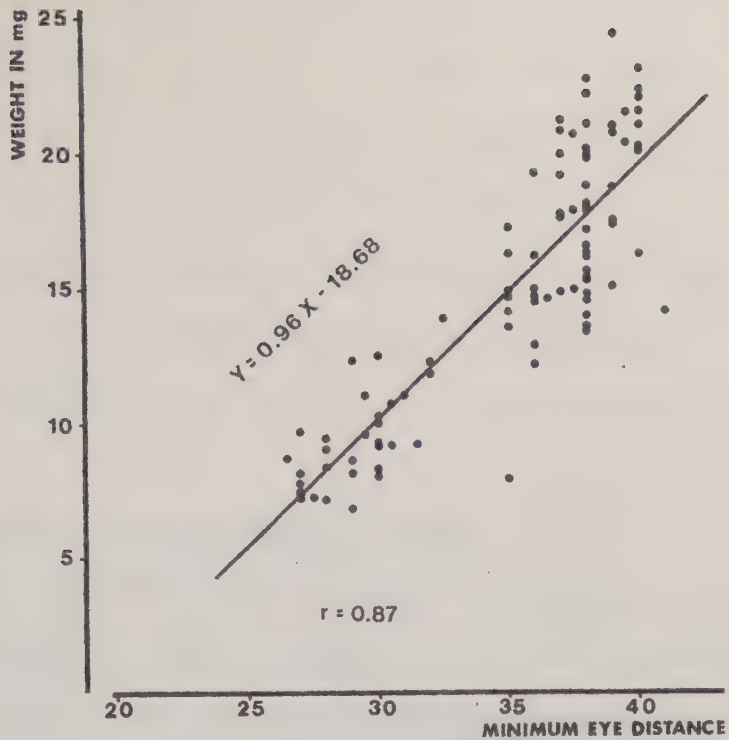


FIG. 3. The relation between weight and minimal eye distance in worker ants of *F. rufa*. The minimal eye distance was measured in ocular measuring units, 30 units = 1 mm.

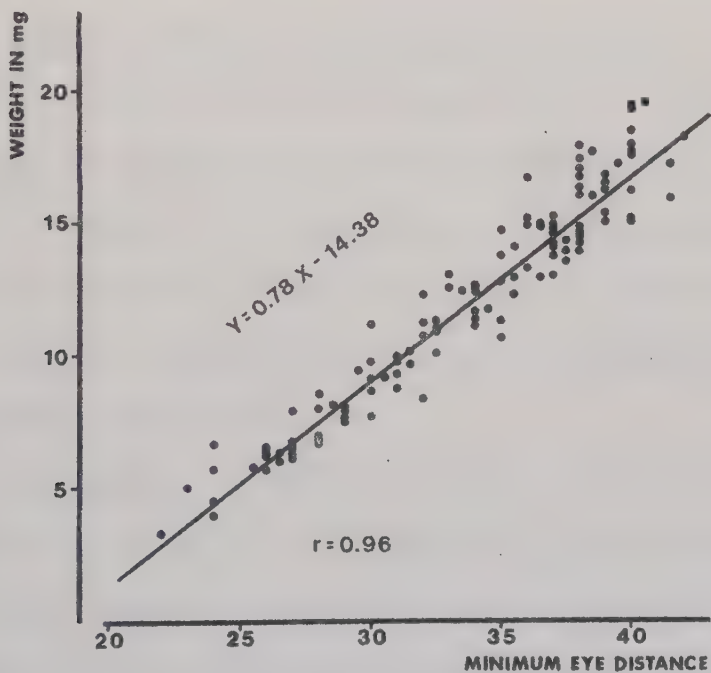


FIG. 4. The relation between weight and minimal eye distance in worker ants of *F. sanguinea*. The minimal eye distance was measured in ocular measuring units, 30 units = 1 mm.

1 μ l, and usually also 0.5 μ l. Formic acid was tested in the same four amounts combined with 0.5 μ l of each of the substance combinations mentioned. The formic acid was always applied on the ants as the second substance. The substances used had a purity of at least 97 %.

On any one day only one amount of one substance was tested on a series of 20-60 ants. The various amounts of a substance were usually tested on consecutive days. Most experimental series were repeated at least once.

Statistical treatment of the material

In many experiments some ants were still alive after the observation time. A mean value for the death-time can for this reason not be calculated. It has been replaced by the median value. The variation can for the same reason not be represented as the standard deviation. A measure of the variation is instead given as two values: The limits for half of the values below and half of the values above the median value.

RESULTS

Natural amounts of defence substances in the ants

The amount of secretion in the poison vesicle in three groups of worker ants of F. rufa was estimated by OSMAN and BRANDER (1961) to be 1.33, 1.09, and 2.81 mg secretion/ ant - mean value 1.57. The concentration of formic acid was on average 63.5 %. Formic acid was found to be almost the only substance in the secretion besides water, these two compounds making up more than 99 % of it (OSMAN and BRANDER 1961).

For F. sanguinea STUMPER (1952) found 0.58 and 0.35 mg of formic acid/ small - medium sized worker ants in two samples. The concentration of formic acid is not known but it is probably about the same as in F. rufa. (OSMAN and BRANDER 1961) determined the concentration of formic acid in four Formica species and found it to vary between 61 and 65 %.

41 substances have been identified from the secretion of Dufour's gland in worker ants of F. rufa (BERGSTRÖM and LÖFQVIST 1973). Two substances, heptadecane and tridecane, predominate. From a gas chromatogram we calculated the

relative proportions in the secretion of hendecane and hendecene to 51.2 % and of tridecane to 19.9 %. It is evident from mass spectra that hendecene is present in one percent or less. The value for tridecane might be too high. All other substances are present in minor or trace amounts only (BERGSTRÖM and LÖFQVIST 1973).

The amount of hendecane has been estimated by gas chromatography of single excised glands to 0.029 mg / worker ant in F. rufa . The corresponding figure for tridecane was 0.007 mg (BERGSTRÖM and LÖFQVIST unpubl.).

The relative proportions between the major substances in the secretion of Dufour's gland in worker ants of F. sanguinea, hendecane, decyl acetate, and dodecyl acetate, were estimated in 1967 by gas chromatography of volatile substances from 324 vacuum distilled ants to 24, 35, and 41 % respectively (BERGSTRÖM and LÖFQVIST unpubl.). These values have been used because they were the only available ones when the present study started. A more precise method has later given the figures 45, 30, and 25 % respectively as results of five gas chromatographic analysis of a single gland in each. The exact amounts were as follows: hendecane 0.026 mg, decyl acetate 0.017 mg, and dodecyl acetate 0.014 mg respectively.

The amounts of formic acid used in this investigation are thus of about the same magnitude as that given off by one or a few ants. An amount of 0.5 μ l of the substances from Dufour's gland, on the other hand, is obviously about 10 times the total gland contents in one ant. Many of these substances have, however, such a low volatility that substances from many spraying ants will concentrate on the body of an enemy.

The implications of the difference between the relative proportions used and the correct amounts is discussed on page .

Toxicity of the substances tested for F. rufa

FIG. 5 and TABLE 1 show the toxicity of hendecane and tridecane and a mixture of the two hydrocarbons for F. rufa. The two hydrocarbons are highly toxic for the ants in the amounts tested. For smaller amounts (1 and 0.5 μ l) the death-time with tridecane is about twice as long as with hendecane (FIG. 5 a). The

two substances have no synergistic effect when mixed in a proportion of 9:1. In accordance with the relative proportions the death-time by the mixture is close to that by hendecane. It is evident that 0.5 μ l of the substances kills between 59 and 70 % of the ants (FIG. 5 c) in a mean time of 197 - 364 seconds (FIG. 5 a, TABLE 1).

The toxicity of the defence substances of F. sanguinea, decyl acetate and dodecyl acetate, and a mixture of hendecane with these compounds in the proportions 24:35:41 was low for F. rufa (FIGS. 5 a, 5 c). One μ l of either of the acetates did not kill a single ant. A mixture including hendecane was slightly more toxic.

The death rate of F. rufa for pure formic acid is 49 % at 5 μ l (FIG. 5 d) - median value for the death-time 607 seconds (FIG. 5 b, TABLE 1) - 18 % at 2 μ l and 0 % at 1 μ l. Pure formic acid has thus a very low toxicity for F. rufa.

The toxic effect of formic acid changes in a drastic way when 0.5 μ l of a hydrocarbon or an acetate was applied to the thorax of the ants and immediately followed by the application of 0.5 - 5 μ l formic acid (FIGS. 5 b, 5 d). This is most pronounced for the acetates which, when combined with formic acid, become a highly toxic defence secretion. The death-times caused by formic acid in combination with an acetate are, however, still much longer than those resulting from a hydrocarbon combined with formic acid. Since the death-time as well as the number of ants killed within 10 minutes by 0.5 μ l formic acid combined with 0.5 μ l of a hydrocarbon show almost the same values as those caused by 0.5 μ l of the two hydrocarbons, the addition of 0.5 μ l formic acid is evidently without appreciable effect for the ants.

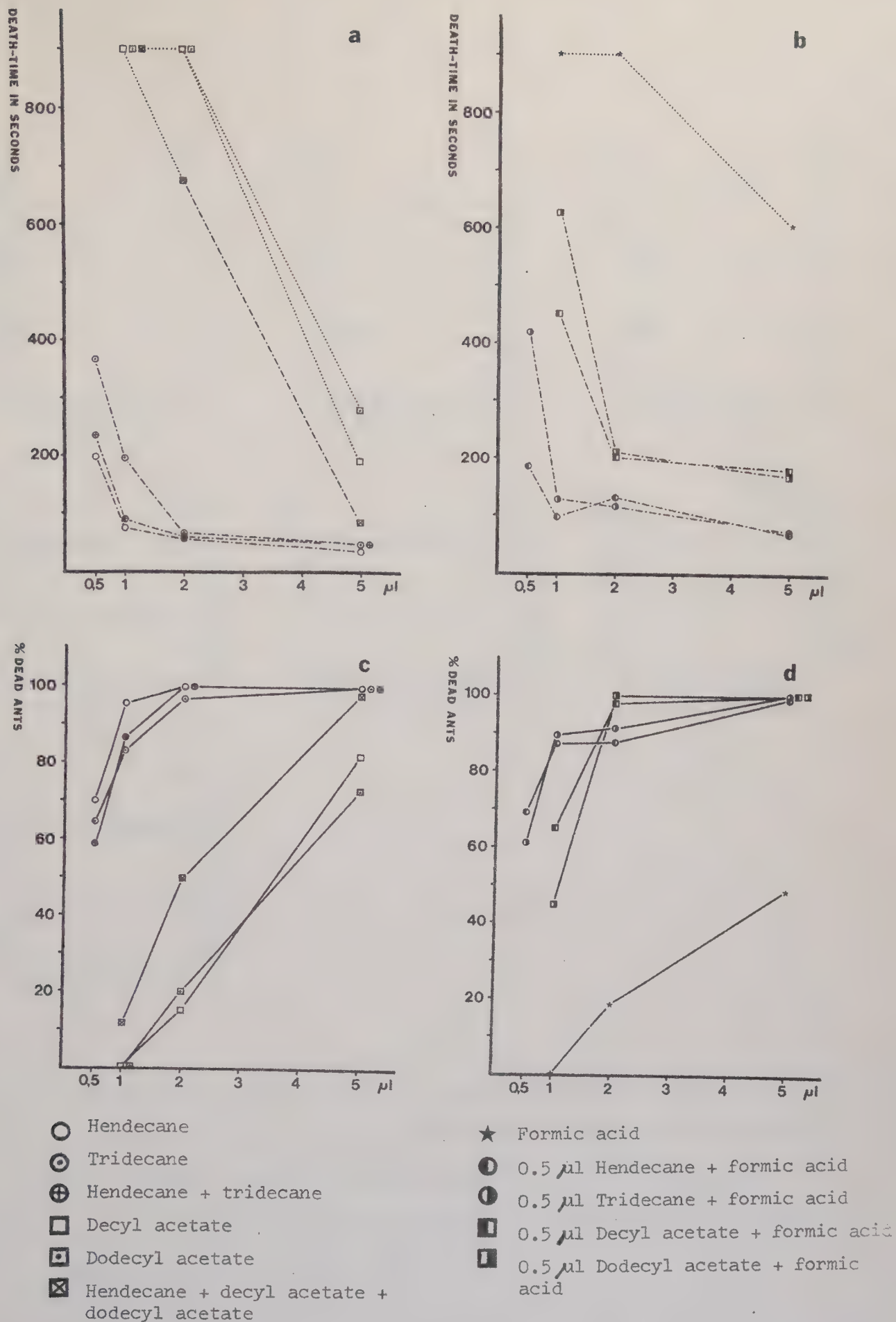
Finally, I tested a combination of formic acid and 1/ 0.5 μ l hydrocarbon mixture and 2/ 0.5 μ l of a mixture of undecane and the two acetates (FIG. 9). The death-times caused by formic acid combined with the hendecane-acetate mixture were more than twice as long as those resulting from formic acid in combination with the hydrocarbons. The percentage of ants killed within 10 minutes was, however, almost the same in the two series. As shown by the distribution of the death-time (FIG. 6) F. rufa is more sensitive to its

TABLE 1 (contin.)

Hendecane + Formic acid	0.5 + 5 0.5 + 2 0.5 + 1 0.5 + 0.5	2 4 3 5	120 240 180 180	37.5 ±1.7 37.1 ±1.5 36.8 ±1.6 36.4 ±1.3	37.7 ±1.7 37.1 ±1.5 37.0 ±1.4 36.3 ±1.2	88.3 131.4 96.8 185.3	99.2 87.9 87.2 69.0
Tridecane + Formic acid	0.5 + 5 0.5 + 2 0.5 + 1 0.5 + 0.5	1 2 2 3	60 120 120 110	36.2 ±1.8 37.1 ±2.0 37.3 ±1.7 37.4 ±1.5	36.5 ±2.1 36.9 ±2.1 37.3 ±1.7 37.5 ±1.3	73.0 116.0 128.3 418.0	100 91.7 89.2 61.3
(Hendecane + Tridecane) + Formic acid	0.5 + 5 0.5 + 2 0.5 + 1 0.5 + 0.5	2 2 2 3	120 120 120 180	37.9 ±1.5 37.4 ±1.6 37.4 ±1.4 37.1 ±1.5	38.0 ±1.3 37.2 ±1.5 37.7 ±1.4 37.2 ±1.5	56.5 64.5 76.0 83.5	100.0 98.0 93.3 87.2
Decyl acetate + Formic acid	0.5 + 5 0.5 + 2 0.5 + 1	2 2 2	100 100 60	38.1 ±1.5 38.0 ±1.5 38.4 ±1.2	38.3 ±1.3 37.9 ±1.4 38.7 ±1.2	181.8 202.5 451.8	100.0 98.0 65.0
Dodecyl acetate + Formic acid	0.5 + 5 0.5 + 2 0.5 + 1	2 2 2	100 100 60	38.2 ±1.6 38.1 ±1.7 38.2 ±1.4	38.2 ±1.5 38.4 ±1.4 38.3 ±1.4	167.3 208.3 627.8	100.0 100.0 45.0
(Hendecane + Decyl acetate + Dodecyl acetate) + Formic acid	0.5 + 5 0.5 + 2 0.5 + 1 0.5 + 0.5	2 2 2 2	100 100 100 80	38.1 ±1.1 37.8 ±1.5 37.5 ±1.8 38.6 ±2.1	38.1 ±1.2 38.0 ±1.5 37.8 ±1.5 39.1 ±1.9	117.0 177.0 240.5 355.5	100.0 99.0 91.0 75.0

Note. The mean value for "Ant size in minimal eye distance" is in the first column calculated for all the ants used in the experiments and in the second column for half the number of ants, i.e. those centered around the median value.

The mean values for "Death-time in sec" and for "Ants dead within 10 min in %" are calculated as mean values of the median values from the various experimental series.



..... The value from experimental series with one of the amounts is longer than the observation time of 15 minutes. The value has been indicated in the figure at the position of 15 minutes.

FIG. 5. Death-times (a, b) for *F. rufa* worker ants during an observation period of 15 minutes and percentages (c, d) of ants dead within 10 minutes after application of test substances. The values are extracted from Table 1.

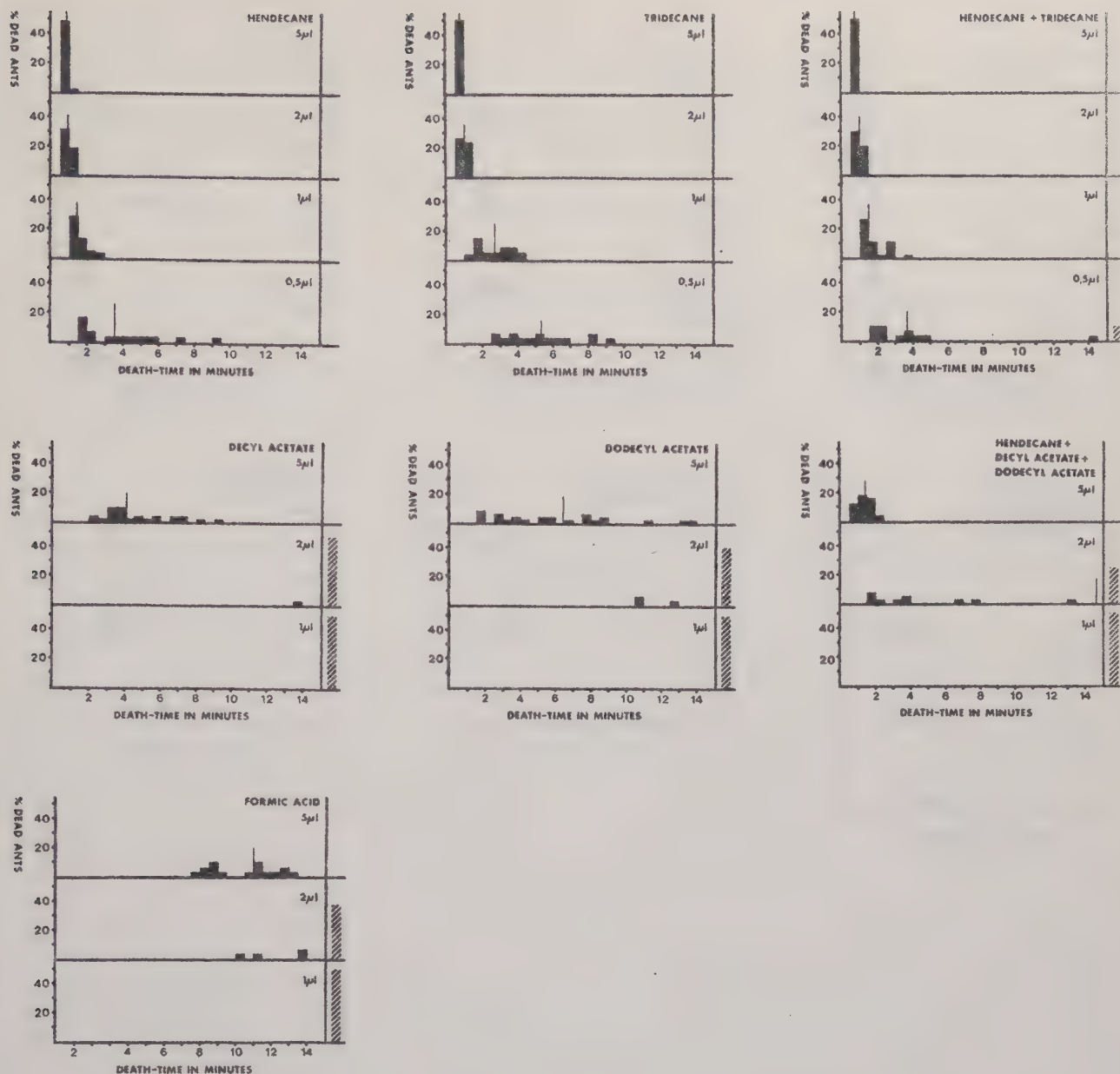


FIG. 6. Single experimental series of *F. rufa* showing the variation of death-times after application of test substances. The thin line in the histograms gives the median value. Each series comprises only the 50 % of the values that are centered around the median value. Longer death-times than 10 minutes are shown as a striped bar.

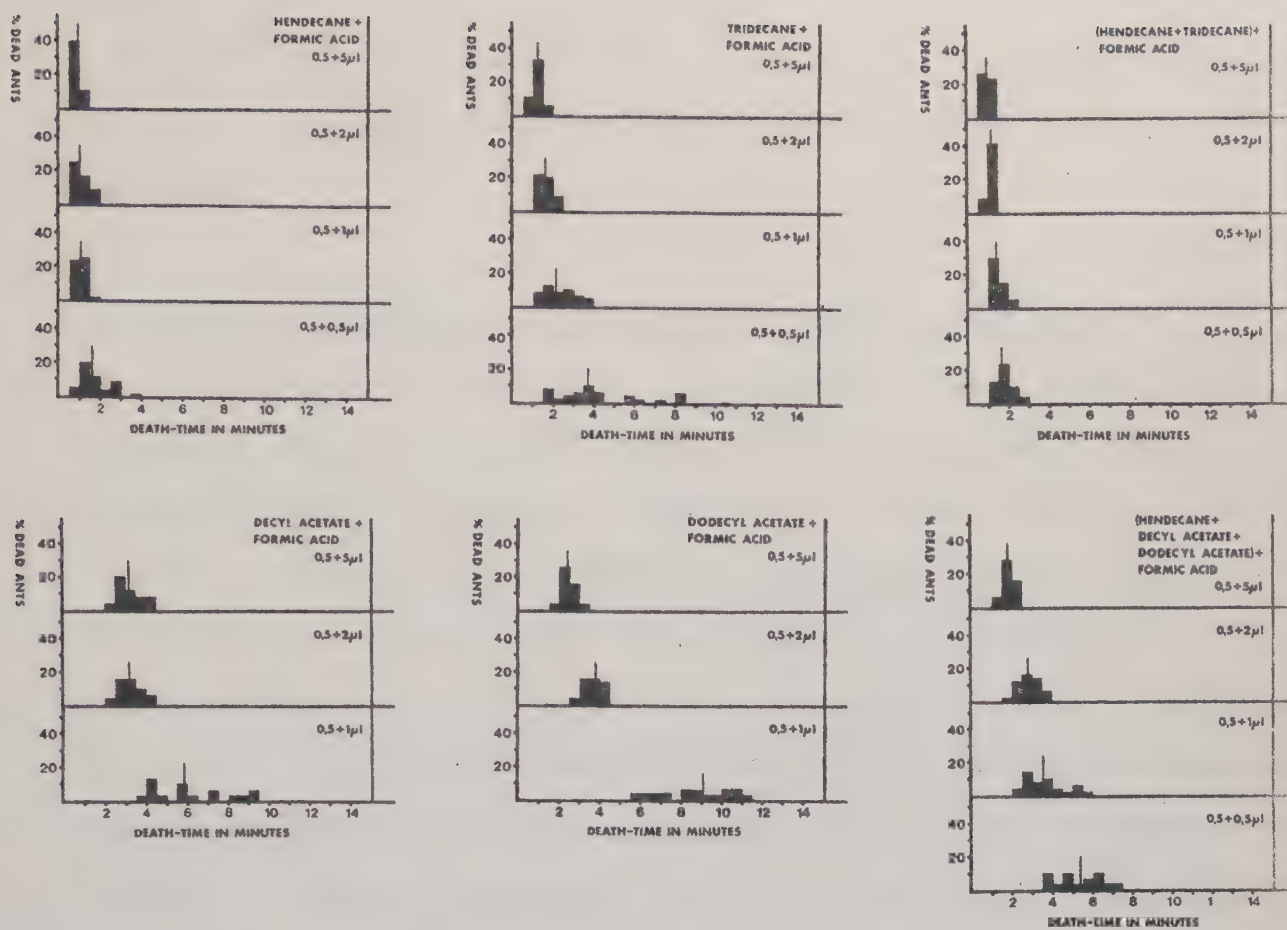


FIG. 6. (Contin.)

own defence substance in the amounts tested here than for those of F. sanguinea. The difference is, however, valid only for the observation period of 10 minutes.

The statistical distribution of the death-times (FIG. 6) was low for highly toxic substances and substance combinations and, as expected, higher for less toxic ones.

Toxicity of the substances for F. sanguinea compared to F. rufa

The same tests were carried out with F. sanguinea (TABLE 2, FIGS. 7, 8) as for F. rufa. In general the results were similar with those from that species. The death-times caused by hendecane, tridecane, and a mixture of the two substances were, however, 2-3 times shorter in F. sanguinea than in F. rufa. The number of ants killed within 10 minutes was higher in F. sanguinea than in F. rufa. The effects were more pronounced for decyl acetate, dodecyl acetate, and a mixture of these two substances with hendecane. The shorter death-times and the high number of deaths in F. sanguinea were found in all substances or combinations tested. F. sanguinea seems to be more sensitive for the various defence substances than F. rufa.

At a combination of formic acid with 1/ a mixture of the two hydrocarbons and 2/ a mixture of hendecane and the two acetates the death-times and the number of ants killed were in the two species very similar (FIG. 9). The death-times in the first-mentioned mixture in F. rufa were, however, shorter than those in experiments with hendecane and formic acid and tridecane and formic acid (FIGS. 5 b, 9 a).

The death-times in experiments with a mixture of hendecane and the two acetates combined with formic acid were shorter in F. sanguinea than in F. rufa. This is in full accordance with the rest of the results.

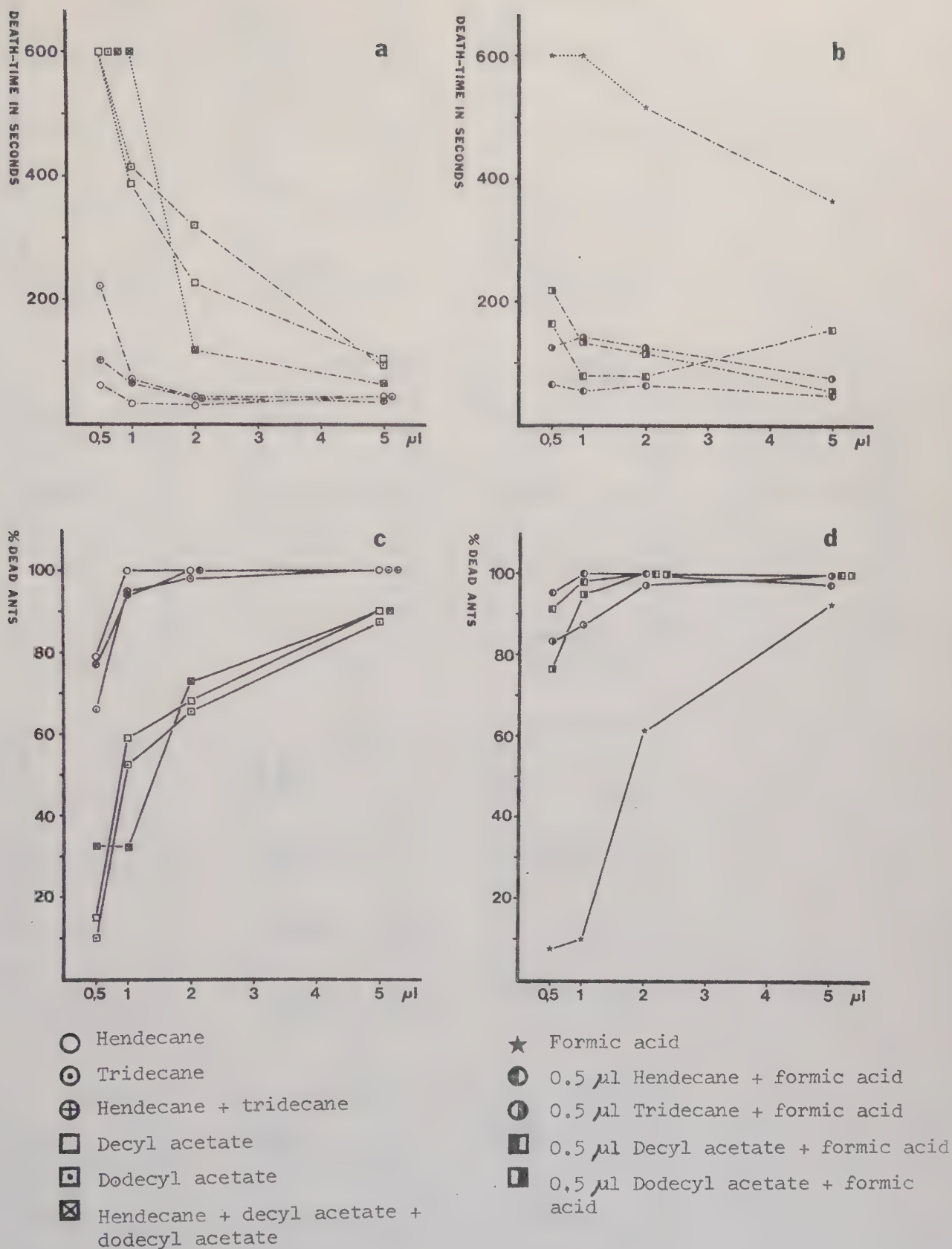
In the mixture of hendecane, decyl acetate, and dodecyl acetate, imitating the secretion from Dufour's gland in F. sanguinea the relative proportion between the substances was 24:35:41 instead of the more correct 45:30:25 (cf. p. 8). The higher percentage of hendecane will result in shorter death-times. The difference between the two species will therefore be smaller than

TABLE 2 - TOXIC PROPERTIES OF SUBSTANCES TESTED ON F. SANGUINEA

Substance tested	Amount in μ l	Number of test series	Σ ants	Ant size in minimal eye distance for		Death-time in sec	Ants dead within 10 min in %
				n_2	$\bar{x}$		
				$\bar{x}$	$\bar{x}_2$		$\bar{x}_1$
Hendecane	5	1	30	45.3 $\pm$ 6.0	44.5 $\pm$ 5.6	45.0	100
	2	1	65	38.6 $\pm$ 3.1	38.7 $\pm$ 2.8	29.0	100
	1	1	65	39.4 $\pm$ 2.7	40.4 $\pm$ 2.0	32.0	100
	0.5	2	105	39.0 $\pm$ 3.1	38.9 $\pm$ 3.1	61.3	79.0
Tridecane	5	1	58	34.2 $\pm$ 4.5	33.8 $\pm$ 4.4	42.5	100
	2	1	50	35.9 $\pm$ 3.6	36.9 $\pm$ 3.0	43.0	98
	1	2	100	33.9 $\pm$ 2.9	34.1 $\pm$ 2.7	70.3	95.0
	0.5	2	100	35.1 $\pm$ 3.0	35.1 $\pm$ 3.1	222.5	66.0
Hendecane + Tridecane	5	2	100	34.8 $\pm$ 2.3	36.1 $\pm$ 2.2	38.3	100.0
	2	2	100	36.4 $\pm$ 2.2	36.6 $\pm$ 2.2	42.5	100.0
	1	2	100	35.4 $\pm$ 2.7	35.3 $\pm$ 2.6	63.5	94.0
	0.5	2	100	37.2 $\pm$ 2.5	37.7 $\pm$ 2.5	102.5	77.0
Decyl acetate	5	2	82	35.6 $\pm$ 4.3	35.6 $\pm$ 4.2	104.8	90.3
	2	2	66	34.1 $\pm$ 4.3	34.3 $\pm$ 4.2	227.0	68.1
	1	2	61	37.2 $\pm$ 2.8	37.7 $\pm$ 2.3	389.0	59.2
	0.5	1	20	37.8 $\pm$ 1.4	33.2 $\pm$ 1.0	>600	15.0
Dodecyl acetate	5	2	80	33.7 $\pm$ 3.8	33.8 $\pm$ 4.1	95.0	87.3
	2	4	140	33.8 $\pm$ 3.2	34.1 $\pm$ 3.3	321.8	65.4
	1	2	40	36.5 $\pm$ 4.0	36.2 $\pm$ 3.7	414.8	52.5
	0.5	1	20	35.0 $\pm$ 1.8	34.9 $\pm$ 1.2	>600	10
Hendecane + Decyl acetate + Dodecyl acetate	5	2	100	35.0 $\pm$ 2.3	35.3 $\pm$ 2.2	65.5	90.0
	2	3	140	34.5 $\pm$ 2.1	34.9 $\pm$ 2.2	117.3	73.0
	1	7	196	34.6 $\pm$ 2.4	34.8 $\pm$ 2.4	>600	32.3
	0.5	4	120	34.4 $\pm$ 2.7	34.4 $\pm$ 2.5	>600	32.5

TABLE 2 (contin.)

Formic acid	5	2	82	38.1	± 3.2	39.1	± 2.4	365.5	92.5
	2	2	65	36.8	± 3.2	36.9	± 3.1	517.3	61.6
	1	2	40	38.1	± 2.2	38.1	± 1.7	>600	10.0
	0.5	2	40	32.7	± 2.1	32.4	± 1.7	>600	7.5
Hendecane + Formic acid	0.5 + 5	2	90	37.1	± 3.1	36.4	± 2.8	48.0	97.5
	0.5 + 2	1	50	35.4	± 3.9	35.6	± 4.1	62.0	100
	0.5 + 1	1	50	35.3	± 4.6	34.5	± 4.7	54.0	100
	0.5 + 0.5	1	42	36.8	± 4.0	36.4	± 4.0	63.5	95.2
Tridecane + Formic acid	0.5 + 5	3	150	34.8	± 2.9	34.9	± 2.8	75.3	100.0
	0.5 + 2	3	150	35.7	± 3.3	35.2	± 3.2	124.7	97.3
	0.5 + 1	4	200	35.9	± 3.2	36.4	± 3.0	142.5	87.2
	0.5 + 0.5	3	130	36.4	± 3.2	36.7	± 2.8	123.7	83.3
(Hendecane + Tridecane) + Formic acid	0.5 + 5	4	190	32.9	± 2.1	33.2	± 2.6	65.0	98.9
	0.5 + 2	4	180	33.3	± 2.3	33.3	± 2.3	78.3	90.4
	0.5 + 1	4	190	33.4	± 2.7	33.3	± 2.8	61.3	89.1
	0.5 + 0.5	5	230	33.3	± 2.5	33.5	± 2.7	78.3	86.0
Decyl acetate + Formic acid	0.5 + 5	2	100	37.9	± 2.2	37.6	± 2.3	156.5	100.0
	0.5 + 2	1	50	35.8	± 3.4	34.9	± 3.5	78.0	100
	0.5 + 1	1	50	37.6	± 3.1	37.1	± 3.0	78.5	98
	0.5 + 0.5	2	82	32.7	± 3.2	31.7	± 3.0	165.8	91.2
Dodecyl acetate + Formic acid	0.5 + 5	1	50	34.0	± 2.6	33.8	± 2.6	55.5	100
	0.5 + 2	1	50	33.6	± 3.7	32.6	± 3.0	115.0	100
	0.5 + 1	1	40	31.1	± 4.4	31.2	± 3.6	136.0	95
	0.5 + 0.5	1	30	32.0	± 4.3	31.6	± 4.4	217.5	76.7
(Hendecane + Decyl acetate + Dodecyl acetate) + Formic acid	0.5 + 5	3	170	34.0	± 2.7	33.7	± 2.5	78.3	100.0
	0.5 + 2	3	170	35.1	± 2.4	35.3	± 2.0	137.0	97.2
	0.5 + 1	4	230	35.4	± 2.4	35.3	± 2.2	176.0	89.1
	0.5 + 0.5	4	210	34.9	± 2.0	34.6	± 1.8	201.1	83.8



..... The value from experimental series with one of the amounts is longer than the observation time of 10 minutes. The value has been indicated in the figure at the position of 10 minutes.

FIG. 7: Death-times (a, b) for *F. sanguinea* workers during an observation period of 10 minutes and percentages (c, d) of ants dead within 10 minutes after application of the test substances. The values are extracted from Table 2.

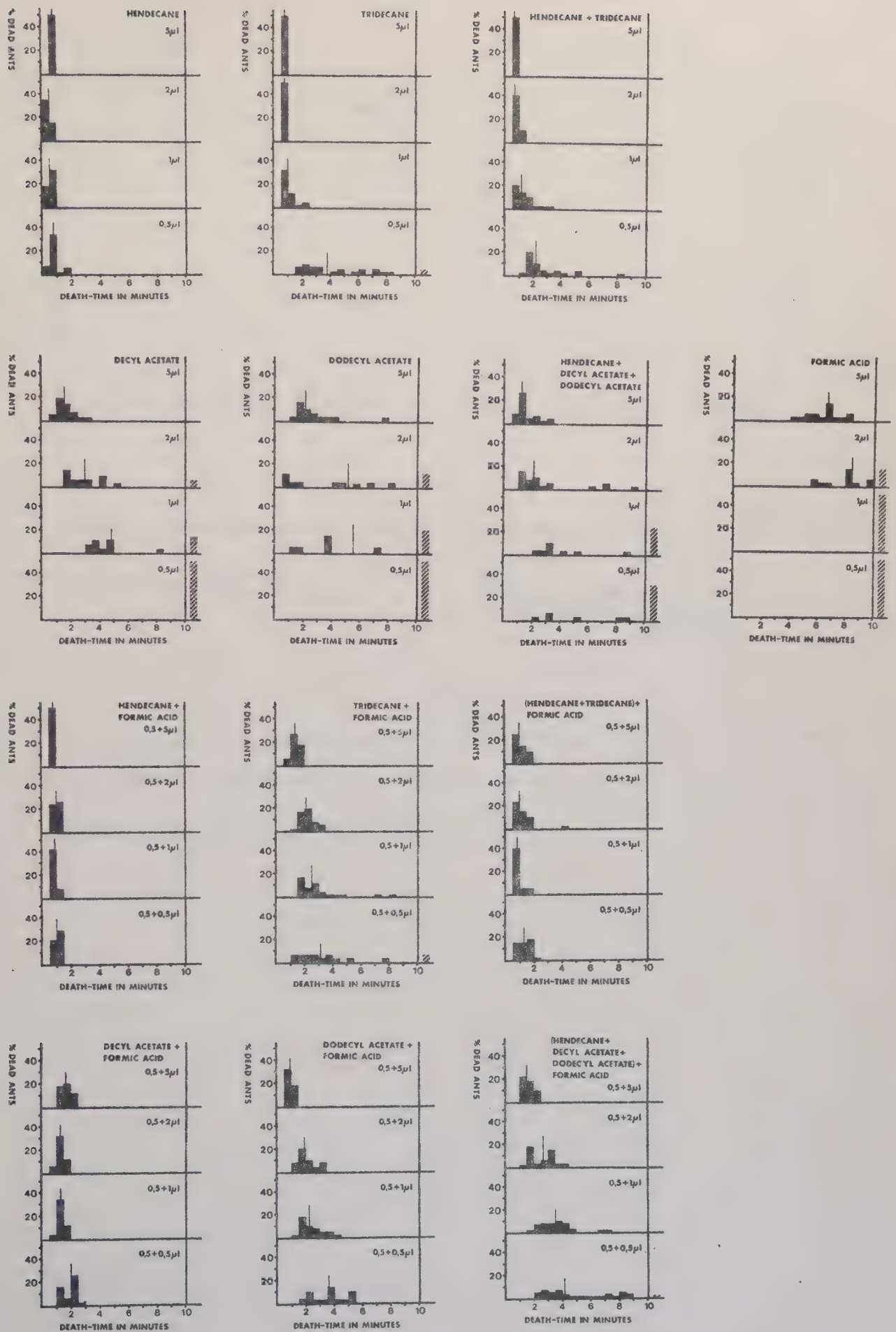


FIG. 8. Single experimental series of *F. sanguinea* showing the variation of death times after application of test substances. For explanations see FIG. 6.

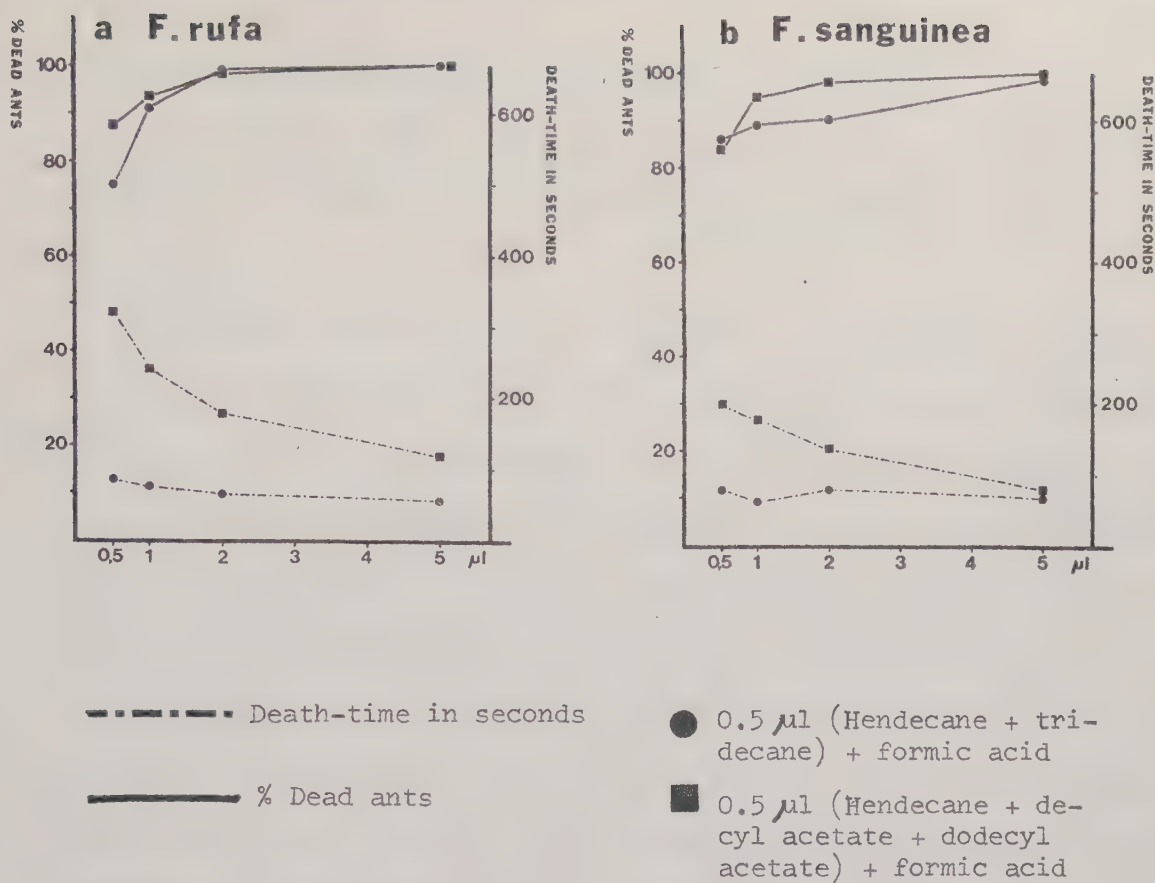


FIG. 9. *F. rufa* and *F. sanguinea*. Death-times and percentages of ants dead within 10 minutes after application of substances imitating the defence substances of the two species. The values are extracted from Tables 1 and 2.

calculated above, though still evident.

Evaporation time and surface tension of the substances

The spreading and evaporation of the test substances determine their mode of action and efficiency. The importance of the factors was studied in some model experiments .

The chemical nature of the outer layer of the integument of formicine ants is unknown. In honeybees a wax layer covers the integument (NEVILLE 1975). This layer was imitated by applying a thin layer of bee wax to glass microscope slides by dipping them in a solution of wax in carbon tetrachloride. The dried slides were treated like the test ants. The same amounts of the same substances and combinations were applied in the same manner on three slides. The evaporation time was measured and the maximal spreading area calculated (TABLE 3). Formic acid is the most volatile with an evaporation time of 30 minutes at 0.5 μ l substance, while decyl acetate and dodecyl acetate have longer evaporation times. The time limits of the tests - for F. sanguinea 10 minutes and for F. rufa 15 minutes - are thus shorter than the evaporation times.

The area over which the test substances spread is about the same in all cases, except for formic acid, where it is about 10 times smaller than for the other substances. An application of 0.5 μ l of a substance followed by addition of formic acid results in a spreading of the acid over the area covered by the substance. Thus, both the hydrocarbons and the acetates act as wetting agents for formic acid. In ants pure formic acid had a low toxicity. A reason is obviously that the acid has a very poor spreading propensity.

There were no differences as regards the wetting properties of the imitated defence secretions between F. rufa and F. sanguinea.

It is clearly visible under a binocular microscope that 0.5 μ l hendecane applied on thorax of worker ants of the two species spread over the whole thorax covering it with a film of the substance.

TABLE 3 - MODEL EXPERIMENTS OF EVAPORATION TIME AND AREA FOR THE TEST SUBSTANCES.

Substance(s)	Amount in μ l	Time in min.	Area in mm^2	Substance(s)	Amount in μ l	Time in min.	Area in mm^2
				Formic acid	5	128,3	6,1
					2	79,0	4,1
					1	50,3	2,5
					0,5	30,0	1,1
Hendecane	5	119,0	157,1	Hendecane +			
	2	58,0	48,2	Formic acid	0,5 + 5	165,0	13,1
	1	36,3	36,6		0,5 + 2	82,0	13,1
	0,5	30,7	16,2		0,5 + 1	62,3	10,4
					0,5 + 0,5	38,0	9,8
Tridecane	5	285,0	125,5	Tridecane +			
	2	211,7	49,0	Formic acid	0,5 + 5	221,7	19,7
	1	126,7	33,0		0,5 + 2	253,3	17,7
	0,5	112,0	12,1		0,5 + 1	240,0	17,2
					0,5 + 0,5	250,0	18,7
Hendecane +				(Hendecane +			
Tridecane	5	118,3	70,2	Tridecane) +			
	2	72,8	41,4	Formic acid	0,5 + 5	182,7	13,1
	1	51,3	20,0		0,5 + 2	106,7	10,6
	0,5	40,3	12,6		0,5 + 1	58,3	8,1
					0,5 + 0,5	35,3	10,6
Decyl acetate	5	>840	77,5	Decyl acetate +			
	2	>840	37,9	Formic acid	0,5 + 5	>300	24,0
	1	>840	22,0		0,5 + 2	>300	26,3
	0,5	726,7	14,7		0,5 + 1	>300	24,5
					0,5 + 0,5	>300	23,8
Dodecyl acetate	5	>1440	146,2	Dodecyl acetate +			
	2	>1440	104,6	Formic acid	0,5 + 5	>2700	32,6
	1	>1440	41,9		0,5 + 2	>2700	29,8
	0,5	>1440	30,8		0,5 + 1	>2700	25,4
					0,5 + 0,5	>2700	21,1
Hendecane +				(Hendecane +			
Decyl acetate +				Decyl acetate +			
Dodecyl acetate	5	>4680	67,4	Dodecyl acetate) +			
	2	>4680	34,4	Formic acid	0,5 + 5	>3000	30,8
	1	>3240	23,5		0,5 + 2	>3000	21,1
	0,5	>1920	16,9		0,5 + 1	>3000	13,4
					0,5 + 0,5	>1960	13,4

Penetration of the substances

Ants of the one species dropped into nests of the other species are killed. Most of them are not visibly damaged. In such cases the defence substances must penetrate the victim's cuticle or reach its vital organs through its tracheal system. In F. rufa ants killed by hendecane stained with Sudan Black B or Sudan Red II the stained substance was found to have passed the stigmata and penetrated into very tiny tracheal branches. No substances seemed to have penetrated an intersegmental membrane or any other part of the cuticle.

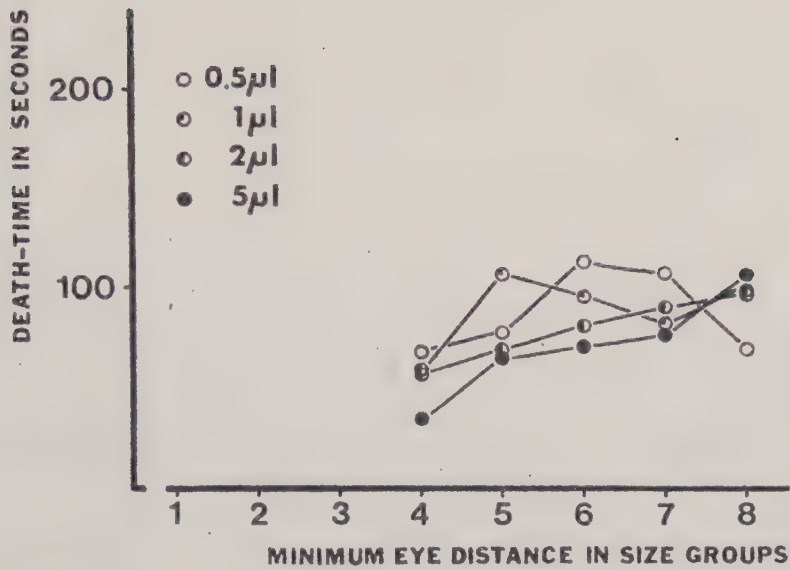
When the tracheal openings on the thorax of F. rufa were sealed with bee wax before the ants were treated with hendecane applied on their thorax the time until death increased considerably. In 29 ants treated with 1 μ l of hendecane each the median value for the death-time was 299 seconds. The corresponding figure for ants with unsealed tracheal openings was 74 seconds. The difference suggests that hendecane normally reaches the ant's interior through the tracheal system.

The importance of the size of the ants

Since the size of the ants was controlled the test material was very uniform (TABLES 1, 2). The size variation was thus of minor importance for the test results. On the other hand, it is of some interest to investigate the importance of the size. This was done for some experiments with F. rufa (FIGS. 10, 11).

The compounds imitating the defence substances of F. rufa are obviously very toxic (FIG. 10). The death-times are short and independent of the amount of formic acid. It was shown above (FIG. 6) that the variation in death-times was very low among ants killed by various amounts of formic acid in combination with a hydrocarbon mixture. It became apparent (FIG. 10) that the size is of minor importance for the death-time.

Formic acid combined with compounds imitating the defence substance of F. sanguinea had a lower toxicity for workers of F. rufa than their own defence substance. This results in a greater variation of the death-times (FIG.



Size group	Size in ocular units	Size group	Size in ocular units
1	26.5 - 28.5	5	34.5 - 36.5
2	28.5 - 30.5	6	36.5 - 38.5
3	30.5 - 32.5	7	38.5 - 40.5
4	32.5 - 34.5	8	40.5 - 42.5

FIG. 10. Death-times among *F. rufa* in relation to size groups. The ants were killed by formic acid combined with hendecane and tridecane. The values are extracted from Table 1. The minimal eye distance was measured in ocular measuring units, 30 units = 1 mm.

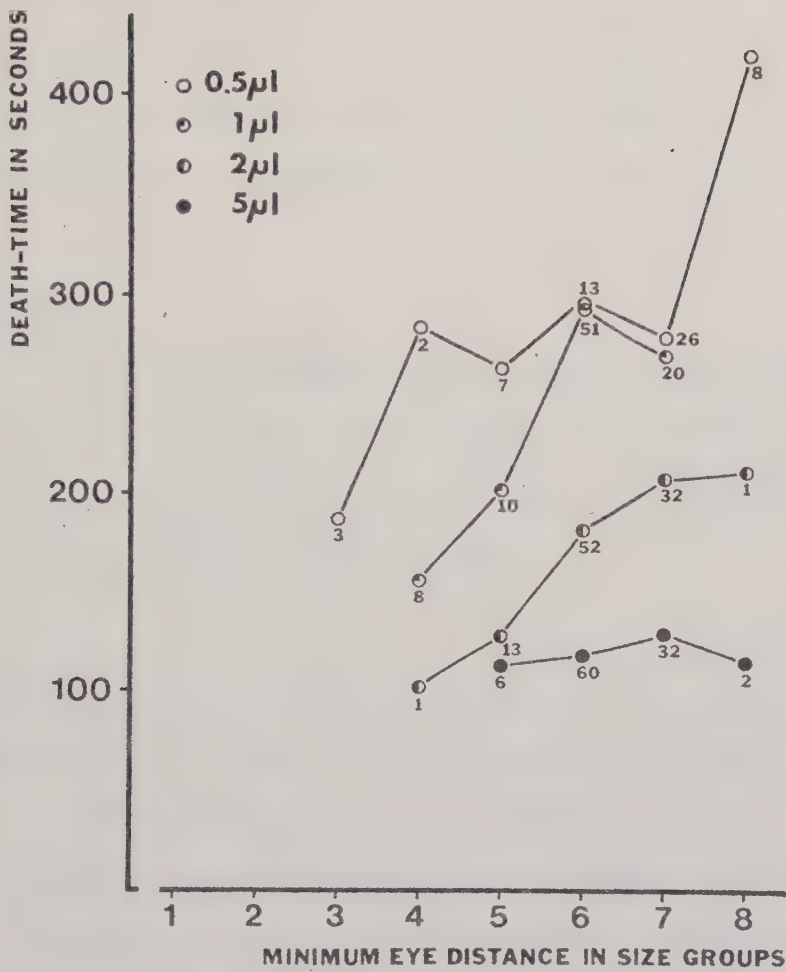


FIG. 11. Death-times among *F. rufa* in relation to size groups. The ants were killed by formic acid combined with hendecane, decyl acetate, and dodecyl acetate. The values are extracted from Table 1. The number of ants in the various size groups is indicated. Size groups as in FIG. 10.

6) and a toxicity increasing with increasing amounts of formic acid. For this combination of substances the size of the ants may be of some importance (FIG. 11). Five μ l formic acid combined with a mixture of hendecane and acetates had such a high toxicity that the size of the ants was without any significance. For smaller amounts of formic acid the size was, however, significant.

The variation of the ant's size was greater and the mean value of the size smaller in F. sanguinea than in F. rufa (TABLES 2, 1). This may explain that in the tests with F. sanguinea the values are always lower than with F. rufa.

DISCUSSION

An evaluation of the chemical defence system in F. rufa and F. sanguinea has to take into consideration the ability of the defence substances a/ to spread out over a hostile insect's epicuticle, b/ to penetrate the enemy's cuticle and c/ the toxicity of the substances.

Spreading

Formic acid is the main defence substance in F. rufa and F. sanguinea. The acid is hydrophilous and on the lipophilous epicuticle it forms droplets which affect a small area only. The substances from Dufour's gland on the other hand are in both species lipophilous and spread out over the epicuticle. As shown above (p.21) these substances promote the spreading of formic acid. There is no difference in this respect between the hydrocarbons of F. rufa and the acetates of F. sanguinea.

The importance of lipophilous defence substances as wetting agents for acids was first shown by EISNER et al. (1961) in the whip scorpion Mastigoproctus giganteus. The defence substance of this species consists of 84 % acetic acid, 5 % caprylic acid and 11 % water. The caprylic acid acts as a wetting agent.

The secretion of the mandibular glands in the formicine ant Acanthomyops claviger contains about 90 % citronellal (CHADHA et al. 1962). Citronellal, like hendecane identified from Dufour's gland in the same species (REGNIER and WILSON 1968), acts as wetting agent for formic acid, facilitating its penetration through the epicuticle (ROTH and EISNER 1962, REGNIER and WILSON 1968).

The amount of hendecane in Dufour's gland in worker ants of F. rufa is about 2 % of the amount of formic acid in the poison vesicle. The total amount of the Dufour gland secretion is about 4 % of the amount of formic acid present. These figures are about the same size as reported by EISNER et al. (1961) for caprylic acid in relation to acetic acid in the whip scorpion. The corresponding figures for F. sanguinea are about twice as high, probably because STUMPER (1952) reported too small an amount of formic acid.

Formic acid sprayed by an ant forms a mist of droplets. The hydrophobous substances from Dufour's gland possibly form a film around the droplets. EISNER et al. (1961) have shown that the spray of the whip scorpion forms droplets each with a volume of less than 0.03 μ l. Such droplets formed by a mixture of 84 % acetic acid, 5 % caprylic acid and 11 % water will spread instantly in contact with the epicuticle of an insect.

Penetration

The defence substances may reach the enemy's vital organs through wounds or by penetrating the cuticle or by following the tracheal system. Penetration of the cuticle may be facilitated by lesions in the enemy's epicuticle caused by the jaws of hostile ants. Most ants killed in fights between F. sanguinea and F. rufa seem, however, to have an intact cuticle.

EISNER et al. (1961) and REMOLD (1962) have shown that the penetration of the cuticle by acids and other lipophobous substances is prevented by the waxy epicuticle. If this is damaged acids can also penetrate the cuticle rather rapidly. EISNER et al. (1961) and REMOLD (1962) showed that lipophilous substances in small amounts promote the penetration of the cuticle of lipophobous substances. REMOLD (1962) also showed that defence substances reach an insect's interior by following its tracheal system. This was confirmed above (p. 23) for ants.

Formic acid is a defence substance not only in formicine ants but also in notodontid caterpillars (POULTON 1888, ROTH and EISNER 1962, SCHILDKNECHT and SCHMIDT 1963) and in carabid beetles (EISNER et al. 1968, SCHILDKNECHT et al. 1968, MOORE and WALLBANK 1968). In the beetles the formic acid is accompanied

by minor amounts of acetates (EISNER et al. 1968) or by hydrocarbons (SCHILD-KNECHT et al. 1968), which may act as wetting agents.

Toxicity

The formic acid as well as the hydrocarbons and the acetates are toxic for the ants when applied to their thorax. The ants are killed more rapidly by the hydrocarbons than by the acetates. The reason for this is unknown.

The chemical defence systems in *F. rufa* and *F. sanguinea*

The main defence substance in both species is formic acid. It is, however, rather harmless except in combination with the secretion from Dufour's gland which acts as a wetting agent. The toxicity of the defence substances and combinations is similar in both species, although *F. sanguinea* usually had somewhat shorter death-times than *F. rufa*. This may be explained by its smaller size. The acetates are less volatile than the hydrocarbons and therefore act as wetting agents for a longer time than the hydrocarbons which may give *F. sanguinea* some advantage over *F. rufa*.

The only difference observed is that the hydrocarbons of *F. rufa* are a little more toxic for both species than the acetates of *F. sanguinea*. Nevertheless, *F. sanguinea* seems to be the superiour ^{nest} competitor in the field (MARIKOVSKY 1963). The reason for this cannot be the difference in the defence substances from Dufour's gland. A possible explanation is that *F. sanguinea* is more aggressive than *F. rufa*. It is well known that there are great differences between formicine ant species in this respect.

The two acetates in the secretion from Dufour's gland in *F. sanguinea* probably have a wide function. REGNIER and WILSON (1971) have shown that acetates from American species of the *F. sanguinea* - group are defence substances, but at the same time serve as alarm pheromones and "propaganda substances" attracting the slave-makers and dispersing the defenders during slave raids. In *F. sanguinea* the acetates are not, however, alarm. pheromones (LÖFQVIST unpubl.). The fact that they are not very toxic supports the view that they are primarily not defence substances. In spite of this they are

almost as effective for the defence as the hydrocarbons in F. rufa.

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